EXPERIMENTAL ZOOLOGY

PART I

EMBRYOGENY

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EMBRYOGENY

AN ACCOUNT OF THE LAWS GOVERNING THE DEVELOPMENT OF THE ANIMAL EGG AS ASCERTAINED THROUGH EXPERIMENT

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WITH SIXTEEN LITHOGRAPHIC PLATES

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PREFACE

THE plan of an *Experimental Zoology* grew out of the revised edition of the *Introduction to the Experimental Morphology of Animals* which appeared three years ago.

The request which reached me from many quarters, that I should add plates and a brief bibliography, and should treat some chapters in greater detail could only be fulfilled by a radical alteration of the "Introduction" and an increase of its size.

Since in the meantime I had written all the chapters at greater length for the University lectures, it occurred to me to base each chapter of the enlarged book on the lectures of a term.

Thus the size of the book grew to be five times that of the "Introduction" and it no longer seemed appropriate to describe it as the "second edition of the Introduction." At the same time the separation of the morphological and physiological experiments proved impossible with the more detailed treatment, and the expression *Experimental Zoology* coined by the Americans in their new magazine has therefore been preferred as a suitable title.

In order that the book may be easily obtained, in spite of the increase in price caused by the addition of plates, it will appear in parts, each of which may be purchased separately. Thus it will be possible to acquire any part which happens to arouse interest without the obligation of purchasing the whole work.

Each part is complete in itself, in so far as this is possible in the case of biological problems intimately connected with one another. At the same time the more rapid appearance of the parts now ready for press will be secured and the disturbing effect caused by lack of continuation avoided.

The five parts, of which this is the first, will appear at intervals of about six months, and will deal with the following problems:

1. Embryogeny: fertilisation, cleavage, organogeny.
2. Regeneration: restitution, morphallaxis, deformation.
3. Phylogeny: specific characters, inheritance, transformation of species.
4. Vitality: colloidal form, growth, movement.
5. Function: action, interaction, adaptation.

The present volume thus deals solely with the first development of the individual organism without regard to its origin, while the problems of inheritance and descent of species will be discussed in Part III., the growth of the developed organism and the relation between the nucleus and cell body in Part IV., the secondary after-growth of lost parts in Part II., and the problems of the maintenance of the functional state, including that of sex, in Part V. A bibliographical list is appended, which includes all works up to the close of 1906.

HANS PRZIBRAM.

BIOLOGISCHE VERSUCHSANSTALT
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[The English translation has been made by Miss Hertha Sollas, and has been revised by Mr R. C. Punnett, of Gonville and Caius College. The author has read the proofs and has made such additions as were necessary to bring it up to date.]

CONTENTS

INTRODUCTION.

If the parts of an animal are examined beneath the microscope they are seen to be composed of smaller units which are termed cells. It is only the very lowest animal forms, the *Protozoa*, which correspond to a single cell. These possess as a rule a single "cell nucleus"; in cases where several nuclei occur they are not separated from one another by cell walls.

In the reproductive process of the *Protozoa* the cell nucleus and cell body divide, and in this way new individuals arise.

Among the *Metazoa* (multicellular organisms) propagation by division occurs only in the lower forms; yet at certain times at least all species of *Metazoa* possess special "germ cells" the material for which is set apart at the first development of the organism, while other cells known as "somatic cells" are devoted to the formation of its body. The assumption formerly made, that among the lower forms of *Metazoa* (sponges, coelenterates) individual somatic cells were capable after differentiation of again becoming germ cells has not been demonstrated (Maas and Brauer).

The male or female germ cells are produced in special parts of the body,—the "gonads." Before their complete detachment they possess like the somatic cells a "proximal" and a "distal" surface (Hatschek, Mark, Rabl).

The nuclei of the male reproductive cells or spermatozoa only contain half as many of the bodies known as "chromosomes," which become visible at certain times, as the nuclei of the somatic cells of the same species [I, 1][1].

[1] Numbers enclosed in square brackets refer to illustrations: Roman numbers refer to plates, Arabian to figures. Figures in round brackets refer to the years in the bibliography.

The female germ cell or ovum must reduce its chromosomes to a number corresponding to that in the spermatozoon by the expulsion of the so-called "polar bodies" [I, 2], and this takes place just before its nucleus unites with that of the entering spermatozoon. At the time of union an equal number of chromosomes is contributed by each parent, and in the fertilised egg the number of chromosomes is again equal to that of the somatic cell [I, 3].

With this act the individual development of the embryo begins.

It is only in rare cases that the development of an embryo from an egg can take place normally without the presence of sperm (Drones, Aphides, Entomostraca). This process is termed "parthenogenesis."

Upon the entrance of the spermatozoon and the union of its nucleus with that of the egg, the process known as "cleavage" takes place, i.e. the division of the mass of the egg by means of incisive furrows into smaller parts known as blastomeres. This process is always preceded by the so-called "mitotic" division of the nuclei which is accompanied by the formation of radiate figures ("astrospheres"). By this process all the chromosomes are divided longitudinally, and a half of each chromosome passes into the nucleus of each blastomere [I, 4—6].

After each cleavage the egg appears as an aggregation of spherical blastomeres, except when the furrows have been prevented by masses of yolk from completing the cleavage. In the interior of this aggregation or "morula," a cavity may arise which converts it into a "blastula" [I, 7].

Now begins the *gastrulation*, in typical cases caused by the invagination of a certain number of the blastomeres into the interior, by means of which the "primitive organs" arise, viz. the invaginated "endodermal" and the non-invaginated "ectodermal" germ layer [I, 8].

The cells of the ectoderm and endoderm are then differentiated in various ways and endowed with special functions. In the ectodermal portion, tufts and rings of cilia and similar structures are generally formed, while the endoderm begins to separate into various "intestinal regions" [I, 9]. Between the two primary germ layers, supporting tissues are formed, either as a mesogloea, a non-cellular mesenchyme (coelenterates), or as a secondarily invaginated "mesoderm" of cellular structure (coelomates).

The ectoderm furnishes in addition the skin covering, including

often the oral and anal invagination ("stomodaeum" and "proctodaeum"), the organs of sense and the nerves; the endoderm furnishes the inner alimentary canal together with its appendages, which serve for respiration and secretion, and so on; the mesoderm furnishes the muscles, connective tissues, and, except in the case of coelenterates, the gonads.

Roux distinguishes two periods, one of organogeny, the other of functional development. In the latter the organs have already attained their specific form and are capable of exercising their functions.

This brief recapitulation of the normal process of development refers chiefly to such forms as pass rapidly through the first period of "organogeny" and at an early stage lead an independent functional life.

Among those forms which remain longer in the egg or in the maternal organism, the stages in which cilia are strongly developed become suppressed, and the early stages of development are generally of shorter duration.

In particular the stages which are known as "larval" may disappear; these are stages of adolescence distinguished by functional "provisional" organs, essentially different from those of the adult form ("imago"). The larvae must undergo "metamorphosis" before they attain their final condition (insects, frogs).

In connexion with complete metamorphosis we are again confronted by the problems of "organogeny," and it is therefore necessary to understand the mechanism of development of animal embryos in the broadest sense, that is, in the case of animals which early become active, up to the stage in which the definitive organs are acquired and perfected for use.

CHAPTER I.

FERTILISATION.

THE first problem in the mechanism of development of animal embryos naturally concerns the cause which determines the transition of the egg cell from a state of passivity to one of continuous development.

This cause the study of descriptive embryology found to lie in the fusion with a spermatozoon, the rarely occurring cases of parthenogenesis being treated as "exceptions."

These exceptions were sufficient to show that any analysis of "fertilisation" which treats "impregnation" as its determinant does not refer the phenomenon to its "final" cause.

In parenthesis we may point out that in many species of animals both maturation processes take place before the entrance of the spermatozoon (sea urchin [I, 10]), in some only one takes place *before*, the other *during* the entrance, (*Amphioxus*, frog [I, 11]), or both may occur *after* the entrance (annelids, gasteropods [I, 12], nematodes). Although therefore in the two latter cases the spermatozoon is a determining cause of the maturation process it cannot be assumed that this is true in general. Even in a form which normally belongs to the last of the two classes quoted above, the egg of the mollusc *Lottia gigantea*, Loeb (1905[6]) was able on the addition of sodium hydrate to sea-water to induce maturation without sperm, and to obtain parthenogenetic larvae.

The brothers Hertwig (1887) were the first to observe that the eggs of the sea urchin, though not normally parthenogenetic, will nevertheless, if allowed to lie for some time, begin to segment and then rapidly die. This indicates that normal "impregnation" serves to accelerate processes of development which would otherwise take

place more slowly. Loeb put forward the hypothesis that in addition to the vital processes, which tend continually to produce the "development" of the egg, "mortal processes" are also taking place. Impregnation produces an acceleration of the vital processes which gives them the ascendancy over the mortal processes in those cases where the egg does not normally develop parthenogenetically. Loeb regards both vital and mortal processes as chemical reactions which may be accelerated by so-called "catalysers." Since the action of such catalysers may be arrested by certain substances ("poisons") in the case of the vital processes Loeb attempted to produce a similar arrest of the mortal processes by the same means. Indeed he and Lewis (1902) were so far able to retard the progress of the mortal processes in unfertilised eggs by means of cyanide of potassium, that the eggs of sea urchins, which would otherwise have lost their power of cleavage after 48 hours, could still be induced to develop, by impregnation or other means, after 168 hours. Less effective was the arrest of the processes by means of cold. Since immature eggs are not liable to perish rapidly, Loeb attempted to discover (1902[3]) whether an acceleration of the process of maturation would also hasten death, and found that starfish eggs are quickly brought to maturity by means of acids and hydroxyl ions, but also succumb to an early death.

R. Hertwig (1896) and Morgan (1896[2]) had shown that the spontaneous cleavage of sea urchin eggs may be promoted by the application of certain substances. But it was Loeb (1899[3]) who by means of methodical experiments determined mixtures of sea-water with certain salts, in which sea urchin eggs develop without impregnation up to the pluteus stage. Delage (C. R. 1907) has recently succeeded in rearing the young past the larval stage.

The substances which prove efficacious are different for different species of animals; in the case of the sea urchin Loeb at first thought that he obtained a specific action from magnesium, but on continuing the experiments (1900) he found that the same effect was produced by increasing the concentration of the sea-water by an equal amount of other salts, indeed even by cane sugar or urea, in place of magnesium chloride. If the sea-water is diluted, no fertilisation takes place (Morgan). Earlier experiments were then called to mind in which other substances had led to "fertilisation" without impregnation. Thus Tichomiroff had stated (1886) that the unimpregnated eggs of the silkworm can be induced to develop by bathing them with

sulphuric acid or rubbing them with a brush; Dewitz (1888) had observed phenomena resembling cleavage in frogs' eggs placed in sublimate, as had also Kulagin (1898) in frogs' eggs treated with diphtheria serum. A temporary reduction of temperature almost to freezing-point also brought about parthenogenetic development in the eggs of the sea urchin, according to Morgan, and in those of the starfish, according to Greeley and Loeb.

Artificial fertilisation has been successfully induced, within the last few years, in a number of animals other than echinoderms; in the medusa *Gonionemus* (Loeb 1901[1]), in the annelids *Chaetopterus* (Loeb 1901[1], Lillie 1904, 1906), *Podarke* (Loeb 1901[1], Treadwell 1902), *Amphitrite* (Scott 1906), *Phascolosoma* (Loeb 1901[1]), in the mollusc *Mactra* (Kostanecki 1904), in *Thalassema mellita* (Lefevre 1905, 1906), in the cyclostome *Petromyzon*, in several fishes (Bataillon 1904) such as *Fundulus* (Loeb 1901[1]), and in many other creatures. The higher the species, the lower as a rule is the stage of development attained. Only certain of the substances employed produce development in any given species.

All the causes hitherto known to produce artificial fertilisation seem to possess in common the power of withdrawing water from the egg; this was suggested by Loeb and confirmed by Spaulding as the result of careful investigation.

It is probable therefore that one at least of the effects of the entering spermatozoon is purely physical, being concerned with the withdrawal of water, and that the union of the sperm with the egg nucleus is not necessary for the further development of the egg. And this may be demonstrated in a more direct manner. After inconclusive experiments made by Piéri (1899), and Dubois (1900), Winkler (1900) succeeded in inducing fertilisation in sea urchins with the extracted juice of spermatozoa, a means which precluded the union of the sperm and egg nucleus. Since the sperm extract remained active after its temperature had been raised to 70° (Winkler 1901) there could be no question of any specific ferment action.

Boveri (1889) impregnated a number of sea urchin eggs with sperm which had been previously immersed in potassium hydrate and found that the nuclei remained separated from one another during the first segmentations, while the sperm nucleus invariably entered one cell only (during the cleavage): a case of "partial fertilisation." Ziegler (1897) placed sea urchin eggs in his "compression-by-current" apparatus and caused them to be driven against threads of

cotton during impregnation; the eggs remain hanging on the threads and are cut through [I, 13]. It may then happen that the sperm nucleus finally remains in one part of the egg, the egg nucleus in the other. In this case the usual changes of the nucleus take place in both parts but only lead to cleavage in the part containing the sperm nucleus, a fact to which we shall return later.

Portions of unfertilised eggs without an egg nucleus also develop in an apparently normal manner after the addition of sperm, as has been shown by Boveri (1896), Delage (1898, 1899), Morgan (1895[3]), for sea urchins and other animals (*merogony* [I, 15]). Some eggs such as those of the sea urchin are extremely plastic shortly after impregnation, so that they can be drawn out into long threads and torn across; if such eggs are first robbed of their envelope by careful shaking (Driesch) and then divided in this way into drops, some of which contain a nucleus and others not, the latter may be again impregnated (1895[5] [I, 16]). In sea urchin eggs which have been artificially fertilised similar experiments in merogony are also successful [I, 17, 18]. In any case there is sufficient evidence to show that neither the presence of the sperm nucleus (cf. e.g. "parthenogenesis") nor that of the egg nucleus (cf. "merogony") nor the union of both nuclei (cf. "partial fertilisation") can be the determining cause of the development of the egg[1].

With these negative results we may combine the positive discoveries with regard to the physical behaviour of the spermatozoon towards the egg. The spermatozoon consists of a more concentrated plasm than that of the egg and contains less water. With reference to this point Waldeyer writes (in Hertwig's *Handbuch der Entwicklungslehre*, Lfg. I, p. 92), "From the chemical point of view we are struck by the great abundance of solid constituents, the presence of which was established by the very first experiments of Vauquelin and Kölliker (quoted from Kühne's *Lehrbuch der physiologischen Chemie*, Leipzig 1868)."

As soon as the spermatozoon enters the egg its nucleus begins to swell up; this, together with the "centrosome" lying in its middle part forms the greater bulk of the "head" which sinks into the egg. The tail which is sometimes present breaks off and remains behind. This process is in complete accordance with the theory that water is withdrawn from the egg.

[1] The necessity of one nucleus will be discussed later with reference to "egg structure"; cf. [I, 14].

J. W. Jenkinson (1904) recently observed in the case of axolotl, that a watery substance forms in vacuoles in the centre of the spermsphere; thus the hygroscopic action of the sperm is actually visible.

The enlargement of the nucleus attained in this way completes thus visibly the homology between the nucleus of the sperm and that of the egg, with the exception of the difference in the arrangement and activity of the "centrosomes." For while the centrosome may also occur in the egg it appears to remain in a passive condition within the nucleus, or it may be absent; that which is brought by the sperm however remains outside the nucleus, produces astrospheres and appears to control the later cleavage.

Ziegler's egg-dividing experiments have already demonstrated this, since only that part furnished with a nucleus was capable of completing the cleavage.

Yet radiations also occur in the other part of these eggs, viz. in the "egg nucleus" part; and in the case of eggs from Ziegler's apparatus where both nuclei came to be contained in one part, radiations are also present in the part without a nucleus. Even in fragments of unimpregnated eggs which are completely isolated and do not contain nuclei, attraction spheres are formed by the means used to produce parthenogenesis (Morgan 1896).

By treating unimpregnated sea urchin eggs with hyoscyamine and nicotine, Wassilieff obtained cleavage as far as the eight-blastomere stage, and here also radiations ("attraction spheres") were active, although they did not contain any centrosomes. With the use of strychnine centrosomes are formed at a later period. In the parthenogenesis induced by magnesium chloride in the eggs of the sea urchin centrosomes are formed at once from the egg (Wilson 1901[5], Wassilieff), even when fragments only of the egg are used; it is thus impossible that a latent centrosome can have been overlooked.

Thus, although the attraction spheres normally proceed from the centrosome of the spermatozoon, we cannot regard the centrosome as the determining cause of the cleavage itself. Its significance in determining the direction of the first furrow will be discussed later.

Another phenomenon which often occurs on the entrance of the spermatozoon is the separation of a yolk membrane. In Loeb's first experiments on artificial parthenogenesis among echinoderms

the larvae differed from those produced by impregnation in the absence of this membrane, in slowness of development and in the fact that they swam at the bottom of the dish instead of rising to the surface, like normal larvae; it is also probable that they possessed but half the normal number of chromosomes.

Herbst (1893) produced the separation of the vitelline membrane in unimpregnated eggs by shaking them whilst immersed in chloroform benzene, toluene, creosote, or oil of cloves, more recently (1904[2]) also with silver treatment. Impregnated eggs which had been deprived of their membrane by shaking produced a second membrane in benzene water; impregnated eggs furnished with a membrane were also able to produce a second membrane.

By the addition of ethyl acetate to a solution which acted by means of increased concentration, Loeb (1905[2]) brought about the separation of the membrane and obtained as many as 100 % of parthenogenetic larvae. Delage had already obtained 100 % of parthenogenetic larvae in the case of the starfish by the use of carbonic acid. According to later experiments made by Loeb (1905[3]), ethyl acetate probably produces the separation of the membrane by the formation of a free acid; for it does not act in a fresh state, while acetic acid proves effective immediately. Hydrocarbons produce membranes during the immersion of the eggs in the solutions, monobasic organic acids and carbonic acid only after the eggs have been transferred to sea-water (Loeb 1905[4]). The higher fatty acids, such as butyric acid, valerianic and capronic acid are even more efficacious than acetic acid.

Simultaneously with the formation of the membrane a great acceleration of the development occurs, and the resulting larvae begin to swim on the surface like larvae produced by impregnation.

In the echinoderm egg the membrane is produced (Herbst 1893) from the clearer superficial layer. According to Loeb its separation may be traced to a secretion of liquid, so that we again perceive a connexion between the formation of membrane and the withdrawal of water from the interior of the egg, followed by cleavage.

The behaviour of the chromosomes does not appear to have been conclusively explained. Delage for echinoderms, and Kostanecki (1904) for *Mactra* have described half the number of chromosomes in parthenogenetic development, as was to be expected, while according to Tennent and Hogue (1906), the number is just as great

as in normal impregnation. But in the latter the chromosomes must be regarded as "bivalent," since one longitudinal half is produced from the pronucleus of the sperm and the other from that of the egg. Doubt has been cast on this interpretation however by the observations of Stevens (1902), and Tennent and Hogue (1906), since both in the case of the sea urchin (*Echinus microtuberculatus*) and also of the starfish (*Asterias forbesii*) the double number of chromosomes may occur normally beside the single number.

In some recent publications (*University of California Publications*, vol. 3, no. 10, 1907) Loeb lays emphasis on the "chemical character of the process of fertilisation" as the starting of oxidation in the right direction.

We may therefore make the following statement, in solution of our first problem:

The cause which determines the transition of the resting animal egg cell to a state of progressive development must be sought in an acceleration of the vital processes which, even in the resting egg, are always going on; and this acceleration is produced in fertilisation, whether artificial parthenogenesis or impregnation, by means of the withdrawal of water.

CHAPTER II.

EGG STRUCTURE OR PROMORPHOLOGY OF ANIMAL EGGS.

ROUX defines "development" as the "evolution of perceptible diversity."

Since we have found that impregnation is not necessary to determine "development," we cannot regard as the cause of the "evolving diversity" the encounter of the spermatozoon with the egg; thus the problem arises as to the manner in which diversity can be produced from the apparently simple egg.

Earlier investigators believed that all the parts of the adult animal must be present in the egg—for example, Bonnet in 1778, after the discovery of spermatozoa in sperm, when even a small huddled figure was supposed to be perceptible in the egg—Hartsoeker 1694, and Dalempatius 1699. The preformation theory, supplemented, in order to explain the origin of several generations, by a theory of "forms within forms" or encasement, fell to the ground when the study of cells and germ layers revealed the processes of development in animals. The theory of "epigenesis" (Wolff 1759) then found favour. This maintained that each successive stage arose anew from the encounter of factors which owed their arrangement to the preceding stage. An undifferentiated egg was supposed to be capable of determining this arrangement.

To this hypothesis objections were raised on theoretical grounds. His brought forward his theory of "organogonal germ regions," which was afterwards supported by the experiments of Roux and termed the "mosaic theory of development." The theory of unequal nuclear division which this investigator at first represented, namely, that the cleavage cells, owing to their reception of different nuclei, are destined to different ends, was afterwards abandoned by him, and

like His he attributed the diversity to the cell body, or "cytoplasm." On the other hand, Weismann, who had published (1893) a *Theory of Evolution*, which was worked out to the smallest detail, sought to retain the "specificness of the nucleus," and to explain by means of supplementary hypotheses ("reserve determinants") the results obtained by experiments in the mechanism of development, which, as we shall see, were incompatible with his theory.

Although it was known, even before experiments had been made, that the eggs of different animals possessed different forms, and that in the same egg different axes and substances might be distinguished, yet to these facts no particular significance was attributed. The geometrical form of the egg was accepted as specific like any other character of the later stages, while the substances in the "cytoplasm" which could be distinguished by colouring, density and so on from the "protoplasm" proper received the general name of "deutoplasm" or "sustenance yolk," and for the greater part still retain this name.

It was only when attempts were made to determine what may develop from each part of the egg (the "prospective potency"—Driesch) that attention was drawn to the possibility of distinguishing in a descriptive manner parts of the egg which normally give rise to some definite formation ("prospective significance"—Driesch). This "promorphology" of animal eggs is adapted to ensure the evolution of diversity during development; we will therefore illustrate it by examples taken from various classes of animals.

1. The egg of *Hydra viridis* [II, 1], our common fresh-water polyp, is at first lobed like an *Amoeba*, and in addition to food granules contains grains of chlorophyll (Kleinenberg) which belong to symbiotic *Algae* and afterwards produce the green colour. The experiments of Hadži (1906) show that *Hydra viridis* may be obtained free from *Algae* if the eggs are formed and constricted off in the dark. The hydras which emerge from such eggs are naturally colourless. Ovocytes of *Hydra* cut in half with a sharp instrument develop again to their normal size.

2. The egg of the medusa *Aegineta* [II, 2], which is nearly spherical, exhibits in all its vectors, according to Maas (1901), a uniform division into two layers of different substance, an inner endoplasm and an outer exoplasm (cortical layer) which traverses the endoplasm as a fine network. This exoplasm afterwards forms the distal surface of the blastomeres. If the eggs are artificially mutilated

the exoplasm still travels to the distal surfaces, so that these surfaces are always covered by it.

3. The egg of the ctenophore *Beroe ovata* is outwardly similar to that of *Aegineta* [II, 3]. The nucleus lies very close to the periphery in the thin "outer" layer and marks the side on which the first furrow is formed. As in the case of the ovocyte, the animal pole is that against which the germinal vesicle rests, so here the animal pole is that against which the nucleus rests. In normal cleavage, after the eight-cell stage has been passed, smaller blastomeres are detached at the opposite vegetative pole; these are the so-called "micromeres," each of which forms one of the well-known "ciliated ribs." If the vegetative part is cut off from the unsegmented egg, the eight ribs are still formed in this part after impregnation (Ziegler 1898, Driesch and Morgan 1895); but if the eggs are divided by a cut running parallel or slightly oblique to the axis given by a line uniting the animal and vegetative pole, a correspondingly smaller number of micromeres and ribs is formed (Driesch and Morgan 1895). We must thus imagine a diversity distributed among eight vectors and afterwards finding expression at the most vegetative point. We shall find confirmation of this view when dealing with experiments on the isolation of blastomeres.

If the animal part is separated it still exhibits complete cleavage, but forms no ribs or stomach. Thus the formative substance of the latter does not extend in the vectors as far as the animal pole.

4. The egg of the sea urchin *Strongylocentrotus lividus* [II, 4] is characterised when mature by a girdle of orange red pigment which extends from the equator of the egg towards the vegetative pole, but does not reach it (Hatschek, Selenka, Boveri 1901). The animal pole, i.e. that near which the nucleus lies, is distinguished by a "micropyle." If the egg is immersed in a solution of Indian ink we at once perceive that the transparent membrane of the egg is pierced at one point by a tubular canal into which the ink solution enters. Boveri, by whom the phenomenon was first observed, shows that this canal takes its origin at the point where the egg is implanted in the germ epithelium. Thus the vegetative pole is derived from the "free pole" of the ovocyte.

The vegetative unpigmented part furnishes the primary mesenchyme and also the larval skeleton, the pigmented zone the intestines and their derivatives, the unpigmented animal part the ectoderm and its differentiations, in the first instance long cilia.

If the eggs are divided before fertilisation and are then fertilised, they produce diminutive but perfect forms, provided the division takes place nearly perpendicular to the ring of pigment, each fragment being then transformed into a small globe with similar layers. On the other hand, fragments which mainly contain the animal part produce "half gastrulas," and at a later stage the differentiations characteristic of the vegetative part, the intestines and so on, are absent. Fragments which are mainly vegetative behave in the opposite manner and produce no long cilia (Driesch 1900[2], 1902, Boveri 1901, Garbowski 1905[2]). The ring of pigment must not be regarded as the substance which determines the organs, but merely as a character, since eggs of the same species which are entirely devoid of pigment, differentiate normally (Boveri, Garbowski, Fischel 1906).

5. A clear division into three regions related to different cleavage cells, was found by Driesch in the eggs of *Myzostoma* [II, 5] a parasite which uses the crinoids as its host and is sometimes assigned to the annelids, sometimes to the arachnoids. The division into regions is independent of gravitation. Experiments have not yet been made with these eggs. In the case of the annelid *Chaetopterus* Lillie (1906) was able by the employment of centrifugal force to compel the different substances of the egg to arrange themselves according to their weight.

6. In many insects the elongated form of the egg determines an egg axis; at one end a micropyle occurs often of complicated structure; in the case of the praying *Mantis* the side which later becomes the "head side" is turned towards the exterior while the eggs are in the cocoon [II, 6].

7. Among the *Mollusca*, e.g. in the case of the snail *Ilyanassa* [II, 7] the vegetative egg pole is formed by a so-called "yolk lobe" which, remaining undivided, always becomes detached during the first cleavage processes and only unites with one of the cleavage cells (Crampton 1906). Of the four larger blastomeres or *macromeres* which are formed first, that with which the "yolk lobe" unites furnishes the primary mesoderm cells. If the yolk lobe is removed before fertilisation the primary mesoderm cells and consequently the band fail to develop although the corresponding macromere was formed.

8. *Dentalium* behaves in a similar manner [II, 9] (Wilson 1904[2,3]). Here a broad band of pigment, olive brown or vermilion red in

colour is present from the first and only leaves a small white dome at the animal and vegetative poles. The ventral dome forms the yolk lobe. If this dome is removed the formation of the yolk lobe is suppressed together with that of the parts dependent on it, namely the post-trochal region, and, strange to say, that of the apical tuft which lies opposite to it. "Vegetative" fragments or those formed by dividing the egg at right angles to the ring produce diminutive but perfect forms.

9. Among the cephalopods (e.g. *Loligo* [II, 9], according to Watasé) all the sides may be recognised from the first, since the elongated eggs are somewhat pointed on the dorsal and flattened on the posterior part. A strictly bilateral cleavage occurs.

10. In the case of the nemertine *Cerebratulus* (*lacteus* [II, 10]—Wilson 1903[1], *marginatus*—Zeleny 1904), before the formation of the polar bodies, a protuberance is visible at the opposite (vegetative) pole which represents the place where the egg was previously implanted. Later on this disappears but the egg axis may be recognised by means of the polar body. Fragments segment, in whatever direction the egg may be cut, like diminutive perfect forms, and produce corresponding "pilidium" larvae.

11. As regards the promorphology or structure of the eggs in the case of *Prochordata*, the tunicates and *Amphioxus* [III, 1], the only plasm structure known in the unfertilised egg appears to be a concentric arrangement of three substances (*Cynthia*—Conklin 1905[3]; the animal pole is marked by the polar bodies. Experiments with unimpregnated eggs are not known to me[1]).

12. Among vertebrates great accumulations of yolk frequently occur and prevent the egg from segmenting uniformly, or at any rate from dividing completely into blastomeres. The part which is comparatively free from yolk then marks the animal (nuclear) pole. In some fishes, the lamprey (*Petromyzon fluviatilis*—according to Herfort) [III, 2], the minnow (*Rhodeus amarus*—according to Ziegler) and the anchovy (*Engraulis encrasicholus*—according to Wenckenbach), the eggs are elongated and the "blastoderm" (the "germ layer") lies at the pole opposite the "yolk." In the case of fish eggs we are really dealing with a nutritive yolk, and not with a substance which determines certain parts (as in the case of *Ilyanassa* for example). This is shown by the experiments of Morgan (1893) on

[1] The experiments on *Ascidia* made by Driesch, and quoted by Maas in his *Experimentelle Entwicklungsgeschichte*, p. 67, seem to refer to impregnated eggs.

Fundulus, in which, by means of pricking, the yolk could be reduced to almost two-thirds of its bulk and yet perfect embryos were produced.

13. It is principally with the eggs of the frog that experiments in the mechanism of development have been conducted (Roux, cf. 1895).

The eggs of the frog (i.e. of the species of *Rana*) are, as is well known, laid in clusters, each egg being separated from the others by a gelatinous covering. The eggs situated in the interior of the cluster are more or less in a position of constraint while isolated eggs or those on the periphery are less subjected to tensions. In each individual egg a distinct polarity may be perceived owing to the presence of two substances, of which one forms the darker animal part, less rich in yolk, and the other the paler, vegetative part with abundance of yolk. The darker part extends along the surface far beyond the equator, so that only a small band remains to represent the paler part, which however is of greater width in the interior. If a recently impregnated egg of *Rana fusca* is thrown into water with its gelatinous envelope, the dark pole becomes directed upwards, the pale one downwards, so that the "egg axis" which unites the animal and vegetative egg poles is vertical, and if the egg is regarded from above the paler dome is completely concealed from view [III, 3]. In *Rana esculenta*, our common water frog, the egg axis is somewhat more oblique, so that seen from above a crescent-shaped piece of the white dome remains visible [III, 4].

We may suppose that we are dealing with two specifically different solid constituents, and the adjustment of position resembles that of the little pith figure balanced by a lead button, the white end corresponding to the weight and the dark to the piece of pith or wood. This was confirmed by the experiments of Roux, who found that fertilised eggs, killed by boiling in water and deprived of their gelatinous envelope, assumed the same oblique position as the living impregnated egg when set floating in a liquid of corresponding specific gravity (water glass, solution of caoutchouc). Small rods cut from the eggs behaved in the same manner and even imitated in form the little toy figure. Frogs' eggs with a gelatinous envelope assume their position by turning within the envelope if they are not in a position of constraint. This mobility is produced at impregnation by the secretion of water from the egg (Schultze) or, if the eggs are laid in the water, by the swelling up of the gelatinous envelope as it enters the water, so that it becomes somewhat detached from the egg. If this violent swelling is prevented by artificial means the

egg is placed in a position of constraint and cannot turn freely within the envelope because its exterior layer adheres to it. Pflüger, who was the first to make these experiments, concluded that since the egg produced the animal and vegetative organs in their normal places with regard to the centre of the earth, the arrangement of the constituents was not a determining cause of its future fate, but that the organism was controlled by *gravitation*. In opposition to this view Roux and O. Hertwig suggested that a rearrangement of the constituents within the egg was still possible, and Born (1894) was able to show by sections that it is in fact only the outermost layer which adheres to the gelatine, while in the interior of the egg the white substance sinks and the darker rises, so that even in the case of eggs in a constrained position the egg axis assumes a position resembling the normal [III, 5].

If the position of constraint is maintained for very long, and the deviation of the axis from the normal position exceeds 70 degrees, a darkening of the white yolk surface thus lying uppermost may be observed even on the exterior; thus the so-called "grey field of Born" is produced.

Notwithstanding the experiments made by Roux (1902), the necessity of gravitation for the normal development of the frog's egg, independent of a promorphological egg structure, has been maintained by Pflüger (1883, 1884), Schultze (1894), Keibel (1902), and Moszkowski (1902). But the fact that it is the egg structure which is the final determinant—to which, as we shall see later, we must add impregnation as a directive factor—was shown by Roux (1905), Morgan (1904), and Kathariner (1901, 1902), by means of the elimination of the effect of the force of gravity.

Roux placed frogs' eggs in an apparatus which caused them to rotate in a vertical plane at so slow a rate that the influence of centrifugal force was scarcely perceptible. The eggs were packed in moist cotton wool which prevented them from altering their position with regard to the horizontal axis. Although under these circumstances the paler ends remained turned in the most diverse directions, thus showing the exclusion of the force of gravity, yet the eggs developed in a perfectly normal manner. In a second experiment Roux caused the eggs to roll freely over one another in a test tube; these "somersaulting" eggs developed normally although their position with regard to the centre of the earth changed almost from moment to moment.

O. Hertwig (1897) subjected frogs' eggs to centrifugal force and produced complete separation of the egg into a simple yolk and a segment containing the "germinal disc" [III, 7]. The former did not segment while the latter produced normal or misformed embryos. An appearance of partial cleavage was thus simulated, such as is normal in eggs containing a great abundance of yolk, for instance those of birds.

While one egg axis is defined with great clearness in the eggs of frogs no further predetermination is known in the "meridians." Yet they possess, like the sea urchin egg according to Boveri's data, a "rotatory structure" [III, 6] as Roux expresses it. All the vectors drawn to the centre from a parallel vertical to the egg axis are (completely—*Rana fusca*, or almost—*Rana esculenta*) of equal value, but from the exterior towards the centre they pass layers of fluid yolk which continually increase in number.

Experiments in removing parts of the unimpregnated frog egg have not been carried out, perhaps on account of the unfavourable consistency of the eggs which easily collapse and perish when injured.

14. Similar experiments have not been made in the case of the hen's egg since fertilisation takes place in the maternal organism. As regards the position of the germ in relation to the force of gravity the case is similar to that of the frog's egg [III, 8]. The germ, which rests upon the roundish yolk, on the longitudinal side of the egg, is always moved so that it remains above. The movement takes place within the egg envelopes (calcareous shell and shell membrane) inside which the egg is held suspended in the extremely fluid white by means of the chalazae inserted in the longitudinal axis of the egg. At one pole of the geometrical longitudinal axis there lies an air chamber.

Hens also lay unimpregnated eggs in which the distribution of the substances exhibits no striking differences from that in the impregnated egg, but as was shown by Barfurth and Lau (1895) they do not possess the power of parthenogenetic development. Thus even in unfertilised eggs conformity to the law of gravity marks an axis at both poles of which heavy masses of specifically different character are accumulated.

Summing up the conclusions furnished by the promorphology of the egg we obtain the answer to our question as to whether diversity can arise from the eggs of animals. Even before fertilisation a structure formed of various substances is present in the eggs, which ensures the evolution of diversity.

CHAPTER III.

DIRECTION OF THE FIRST CLEAVAGE FURROW.

Our next task is to determine the direction of the first furrow, a problem propounded by Roux (1883), and simultaneously and in a similar manner by Pflüger.

Roux' observations and experiments refer in the first instance to the frog's egg, which we will therefore consider first, following the recent description by this author. The frog's egg (*Rana fusca*) is divided by the first furrow into two parts of equal size; this accords with the first principle of cell division as formulated by the botanist Sachs, namely, that cells tend to divide into two equal parts. The mechanism of mitotic cell division in general will be discussed later; for the special case of "equal division" no determining factor is necessary beyond an approximate symmetry between the divided form and the plane of division.

In the frog's egg, as we have seen, this is the case when the first furrow lies in any one of the meridians, i.e. passes through the animal and vegetative poles and has the egg axis for its diameter. In fact we see the first furrow start normally from the animal pole, and run meridionally to reach the vegetative pole. This is due to the fact that it has been preceded at the animal pole by the nucleus which has already accomplished its division.

Thus the promorphological structure of the egg precludes any coincidence between the equator and the first furrow, but in consequence of the rotatory structure of the egg [III, 6], explained in the preceding chapters, there remains the choice between all the meridians, since in the unfertilised egg these appear to be of equal value as regards their constitution.

If fertilisation is to produce any change in these conditions it would be natural to make the entering spermatozoon responsible in determining which meridian shall be chosen for the first furrow.

Roux terms the "point at which the spermatozoon enters the yolk" the "fertilisation point," and the side of the egg at which it enters the "fertilisation side" of the egg. The meridian containing the point of entrance has been termed the "fertilisation meridian."

The fertilisation point remains visible owing to its deeper pigmentation. On the side opposite the fertilisation side the dark cortical layer of the egg becomes paler, so that even in the egg of *Rana fusca* seen from above a pale crescent-shaped part is visible; this is "Roux' grey field" [III, 11] which must not be confused with "Born's grey field" since it presents an opposite position and mode of development.

Normally the sperm- and egg-nuclei now meet, according to Roux, in the plane of the fertilisation meridian, which in this case may also be termed the "fertilisation plane" [III, 9].

In 75 per cent. of the cases the first furrow lay in the fertilisation meridian [III, 9, 10] and in the remainder *near* it, when the eggs were associated with spermatozoa without any further precautions.

Roux however obtained a much more definite result by an arbitrary determination of the fertilisation point.

The sperm was conducted up a silk thread to a point chosen at random in the egg, which it was only able to enter along a meridian made more easily accessible by means of an incision in the gelatinous envelope. In this case the first furrow always coincided with the fertilisation meridian which was determined by the incision.

The fertilisation plane or first furrow divides Roux' "grey field" into two halves. Roux regards the depigmentation of this field as a process of rearrangement. In any case we see that the fertilisation meridian, which may be artificially determined, produces a rearrangement in the materials of the egg corresponding to a fresh symmetry. The possibility of determining this meridian artificially once more demonstrates that a "rotatory structure" exists before impregnation.

In this connexion we may also mention that the polar structure of frogs' eggs becomes more marked at the approach of the sperm, and before its union with the egg, the specifically lighter formative yolk accumulating more densely beneath the brown cortical layer than was previously the case. Roux supposes that materials are diffused in advance of the sperm head, which determine an alteration in the consistency of the egg. Impregnated eggs, floating freely, turn, together with their gelatinous envelope, so that the pale

pole is downwards; eggs prevented from floating freely by the adherence of the gelatinous envelope require several hours to turn if unimpregnated, if impregnated only half an hour, a fact which must be ascribed to the sharper separation of the specifically different heavy substances.

In order to obtain fixed points in the experiments Roux made use of small pieces of hair which were stuck into the gelatinous envelope.

From the more recent descriptive observations of other investigators in the case of many kinds of animal eggs Roux subsequently generalised the law discovered in the case of the frog's egg for the typical occurrence of the first furrow:

"In round eggs with an indifferent structure, that is, of many axes, or possessing a yolk of rotatory structure and of one axis (like the cell body of the egg), the yolk and particularly the nutritive yolk (protoplasm) guides the entering spermatozoon in such a way that it proceeds in the plane of a meridian (= great circle) passing through the point of entrance, or, in the second type of egg quoted above, in the plane of the meridian passing through the point of entrance and the egg axis. The first division of the yolk then takes place in the copulation direction of the cleavage nucleus, in the copulation path of the sexual nuclei in the yolk, and in the fertilisation meridian, since all three lie in the same plane.

"A second case concerns eggs which are not round as well as round ones, which before fertilisation present an arrangement of the yolk materials essentially different from the rotatory structure and retain it during fertilisation. This case includes those eggs in which such a deviation may be produced by exterior means such as pressure or, in the case of eggs in a constrained position, by gravitation. In this case the yolk acts on the spermatozoon to a degree dependent on the position of the fertilisation point with regard to the yolk structure, in such a manner that it diverts the spermatozoon more or less from the fertilisation meridian; it may even exert a turning force on the already copulating sexual nuclei and on the cleavage nucleus. In this way the copulation and divisional direction of the cleavage nucleus is made to coincide with the nearest direction which is mechanically suitable for the division of the yolk."

That the spermatozoon is diverted from its course in fertilisation may be clearly demonstrated by means of the band of pigment which the sperm head leaves behind it.

In typical normal eggs this band lies throughout its length in the plane of the first furrow.

In exceptional cases, however, when the position is one of constraint, the band of pigment turns from the "penetration path" which at first lies radially in a median plane and hastens towards the (displaced) egg nucleus [III, 10]. In such cases the last part of its course, the "copulation path," determines the meridian in which the first furrow occurs. After impregnation the egg nucleus also makes a movement towards the sperm nucleus, and in the case of the sea urchin (*Toxopneustes* = *Strongylocentrotus*), according to Wilson, it follows in a somewhat circuitous manner the "copulation path."

The nuclei flatten out against each other after coming into contact, and the surface thus produced is known as the copulation surface (O. Hertwig). On the periphery of this surface occur the poles of the nuclear spindle, the axis of which thus comes to lie in the copulatory surface. The division of the nuclear mass then takes place at right angles to the nuclear spindle, followed by that of the cytoplasm (cell body) in the same direction; this law was formulated by O. Hertwig for mitotic cell divisions in general. By means of a particular case it furnishes the explanation of Roux' principles, by which the first furrow is determined by the position of the copulation path when the copulating nuclei have not undergone rotation.

This law refers to the typical and normal cleavage of round ("rotatory") eggs. In *Toxopneustes* Wilson also observed (1901) that the united nuclei assumed an almost central position and this again corresponds to a general rule formulated by O. Hertwig:

"The nucleus tends to take up a position in the centre of its sphere of influence, i.e. of the protoplasmic mass in which it lies."

In the elongated egg of *Ascaris nigrovenosa* the copulating nuclei rotate in such a way that the axis of the nuclear spindle, which originally lay in one of the shortest axes of the egg, now lies in its longest axis, that is it has passed through an angle of about 90°. Thus in this case (O. Hertwig) the first furrow, which is perpendicular to the axis of the spindle, is produced as an equatorial furrow.

O. Hertwig has stated as a general principle that the axis of the nuclear spindle tends to lie in the longest axis of the protoplasmic mass, so that a division perpendicular to this axis must divide the cell in approximately spherical parts, i.e. cells without a strongly marked axis. Experiments in compression and elongation by which an egg of one axis (frog or sea urchin) was compelled to assume

a new geometrical longitudinal axis, have shown in the first place that the first furrow assumes a perpendicular position with regard to vertical glass plates exerting pressure upon the egg (Roux, Born 1894 [III, 12—14]), that is the nuclear spindle has remained perpendicular to the axis of the egg structure but must have rotated from the fertilisation meridian to that meridian which was compelled to assume the shortest geometrical axis in the equator.

On the other hand Roux introduced frogs' eggs by suction into glass tubes of smaller diameter than that of the egg, and found that when the structural axis was considerably elongated the first furrow stood perpendicular to the axis and to the glass sides, that is the nuclear spindle had turned so that it lay in the structural axis which was now the longest axis [III, 15].

By using tubes of *greater* diameter an elliptical deformation of the eggs, adhering to the glass sides, was sometimes produced; here the first furrow was formed sometimes parallel to the glass sides, sometimes, but more seldom, perpendicular to them [III, 16].

There are, according to Roux, two positions of equilibrium for the nuclear spindle, namely the largest or the smallest (the latter possibly labile?) geometrical axis of the egg [III, 17].

Boveri (1901) in his experiments on elongation and pressure of sea urchin eggs, likewise observed a competition between the structural axis and the enforced geometrical axis, which in this case generally caused the nuclear spindle to assume an oblique position and consequently also the first furrow, which stands perpendicular to it.

Certain investigators (Michaelis, O. Hertwig) raised the objection that so great a mutability in the mode of occurrence of the first furrow was incompatible with Roux' theory that bilaterality was determined by the fertilisation meridian; but recent investigations made by Brachet (1906) on the frog show that the bilaterality does not in fact appear until after impregnation. Brachet observed a compensation of all defects in the frog's egg within 45 minutes after impregnation, afterwards complete bilaterality appeared, and this, without regard to the particular course of the furrows, produced the final bilateral symmetry of the embryo.

According to the investigations of Conklin (1905[3]) on the egg of the tunicate *Cynthia partita*, the concentric arrangement of the three layers of the unfertilised egg changes with maturation and impregnation. The yellow substance on the surface flows towards the

vegetative pole (cap). Above it rests the transparent fluid which escapes from the germinal vesicle, and in this way a polar radial arrangement is produced. The sperm nucleus lying in the middle of the yellow dome draws the transparent and yellow substances out of the vegetative pole; the yellow substance assumes the form of a crescent with its centre at the further pole and the transparent matter comes to lie above it. Thus the position of the sperm nucleus determines a bilateral symmetry and the first furrow appears in the plane of symmetry. When the cleavage is complete the clear substance occupies the animal (ventral) half of the egg, and the original middle grey layer the middle of the vegetative (dorsal) pole, while around the posterior margin of the dorsal hemisphere lies the yellow crescent, and around the anterior margin the pale grey crescent. This final localisation shows that the clear substance produces the ectoderm, the yellow crescent the muscles and mesenchyme, the grey crescent the chorda and neural plate, and the grey substance the endoderm. Lillie describes a similar alteration of egg structure in the annelid *Chaetopterus*.

We thus arrive at the result that the direction of the first furrow is given by a plane perpendicular to the axis of the first nuclear spindle, and the position of the latter appears as the resultant of egg structure, geometrical form, and fertilisation meridian. It may be determined in the cases specifically investigated in different eggs by ascertaining the particular values of these factors.

CHAPTER IV.

MITOTIC CELL DIVISION.

(a) *Migration of the nucleus.*

BEFORE tracing further the history of the two blastomeres separated by the first furrow we will give some account of the mechanism of cell division in general. We have already heard of the "spindle of nuclear division," which determines the direction of the division. The question now arises as to what forces determine the production of such a spindle, and what is its relation to the division of the nucleus and of the cell body.

We must first describe in some detail the mitotic divisions of the nucleus and the cell as they have become known to us from recent cytological researches [IV, 1—10].

These researches show that the resting cell possesses in addition to the cell body and cell nucleus an important organ known as the centrosome. This consists of a minute body of high staining capacity (or more rarely of several such bodies) surrounded by a spherical mass of which the limits are not clearly defined.

When the cell is preparing to divide, the sharp boundary of the nucleus disappears and the staining mass of the nucleus consisting of the so-called chromosomes becomes strongly marked.

The centrosome divides and the parts diverge to opposite sides of the mass of chromosomes, a process accompanied by the formation of astropheres. The chromosomes, previously a confused knot (spireme) now become arranged in a plane (equatorial plate).

When the centrosomes have reached a position opposite to one another they are united by the so-called nuclear spindle, composed of achromatic rays running from each centrosome to the several chromosomes formed by the transverse division of the spireme thread. Now the chromosomes divide longitudinally and move

apart in the direction of the centrosomes. The breaking up of the nuclear mass is followed normally by a constriction and division of the cell body.

The nuclear masses of each of the new cells now move towards the middle of the cell (O. Hertwig's law) and become transformed into resting nuclei with a sharp boundary, while the radiations disappear and the chromosomes are no longer visible.

The next division of the nucleus and the cell is again preceded by the division of the centrosome, the parts of which again tend to assume a position opposite to one another.

Zur Strassen, who has made the development in the egg of the threadworm, *Ascaris*, his basis of observation, points out that the centrosome lies near the free surface of the cell. In *Ascaris* the conditions are favourable for observation since the "sphere" surrounding the centrosome remains visible even in the resting cell.

When a cell divides it is obvious that one of two cases may present itself; either the division takes place so that the new centrosomes in the new cells lie near the free surface in their typical position, or they appear to approach a plane of contact. In this case, according to Zur Strassen, a migration of the centrosome takes place until it again reaches the free surface, and in particular the pole of the symmetrical axis ("form axis") determined by the planes of contact [IV, 11]. Thus if the symmetrical axes of two adjacent cells, which have just been produced by the division of a single cell, make an angle α, then each centrosome in order to reach its resting place must describe an arc $R - \frac{\alpha}{2}$ [IV, 12]; that is to say the value of the angle of movement increases as the divergence of the symmetrical axes decreases. Under these circumstances the arc which must be described in a closed epithelium, the symmetrical axes of which scarcely diverge at all, amounts to about 90° [IV, 14], in the four-cell stage to about 45° [IV, 13], in the two-cell stage where $\alpha = 2R$ and $R - \frac{\alpha}{2} = R - \frac{2R}{2} = 0$, no displacement occurs.

The rôle which the centrosome plays in the division of the cells, led to its designation as the "dynamic centre" of the cell (Boveri). In this connexion the fact was quoted that in *Ascaris* the astrospheres are formed about the centrosome which is introduced by the sperm into the egg cell, whereas no centrosome appears to be present in the mature egg cell, or at any rate is not concerned in the process

of division. The case is similar in the sea urchin egg, where the centrosome of the egg cell remains inactive in the nucleus, while the radiations proceed from the centrosome which has been introduced in the middle part of the spermatozoon.

An excess of motive energy, residing in the centrosome of the spermatozoon, was thus believed to determine the development which first becomes manifest in mitosis. This view does not accord with the observations of Van Beneden and Wheeler on the eggs of *Myzostoma*, where it is definitely the egg centrosome that exhibits clearly marked radiations and is said to accomplish the division. According to Kostanecki however this is not certain; both in this case and in that of the snail *Physa* radiations are formed by the centrosomes; at a later stage however the attraction sphere of the egg degenerates so that it is still the middle part of the sperm which furnishes later the starting point of the mitotic figures. Thus normally the centrosome would be derived from the spermatozoon in the case of the cleavage cells and, at a later stage, also in that of the somatic and sexual cells.

Nevertheless a pre-formed centrosome is not indispensable for the processes of development and the mitotic divisions, as is shown by the cases of artificial parthenogenesis discussed above, in which no sperm centrosome is introduced and egg centrosomes only appear in a typical manner after the first divisions, that is, are formed anew.

That we are really dealing here with a new formation is proved by the experiments of Morgan (1896[3], 1899, 1900) who placed the unimpregnated eggs of echinoids (*Arbacia*) and other animals (*Asterias, Sipunculus, Cerebratulus*) in weak solutions of common salt or magnesium chloride and observed that several radiations appeared in one egg. These astrospheres exhibited centrosomes in their centres which could not be distinguished from the normal forms. They showed a high affinity to stains, and wherever two were associated together they began to develop nuclear spindles and to divide the nuclear mass.

By cutting pieces of protoplasm from eggs of *Cerebratulus lacteus* and treating these fragments, devoid of nucleus with potassium chloride, Yatsu (1905) obtained asters, centrosomes and centrioles, but only after the germinal vesicle had disappeared and the nuclear substance had begun to disperse.

A number of mitoses may also arise when several spermatozoa enter one egg, a phenomenon known as polyspermy. Normally the

egg membrane which is separated after the entrance of the first spermatozoon prevents the entrance of all further spermatozoa. Immature eggs, however, or those weakened by treatment with various reagents (nicotine, etc., O. and R. Hertwig 1887) as well as those "hybridised" with sperm of another species, permit the entrance of several spermatozoa without the separation of an egg membrane.

Radiations now arise about each sperm centrosome, every two adjacent centrosomes together producing an astrosphere. If fertilised eggs are placed in salt solutions (Morgan 1899), the division of the nucleus and protoplasm is retarded. Artificial astrospheres are also present in these eggs and participate in an irregular distribution of the chromosomes.

In every case the division of the yolk depends upon the position of the newly formed nuclei but otherwise takes place without any relation to the number and position of the astrospheres.

The observations of Boveri and Ziegler also demonstrated that the presence of the nucleus is necessary to produce division of the yolk, while the attraction spheres are powerless if the nucleus is absent. Boveri observed that enucleated fragments of the eggs of *Echinus microtuberculatus*, if impregnated with the sperm of *Strongylocentrotus*, i.e. hybridised, divided so that the impregnated nuclear substance passed into one of the cleavage cells, which then segmented regularly, while in the other the centrosomes and attraction spheres continued to divide but the cell failed to do so. Ziegler (1897) observed the same phenomenon in the non-hybridised egg of *Echinus microtuberculatus*, but constrictions were indicated in that part of the egg devoid of chromosomes, and irregular and feeble segmentations afterwards occurred. The importance of the nucleus for the regular division of the cells by means of astrospheres is also emphasised by N. M. Stevens (1902) and Teichmann (1903). Complete division of the cell however does not necessarily follow upon division of the nuclei. E. B. Wilson (1901[2]) found that sea urchin eggs (*Toxopneustes variegatus*) when acted upon by ether only developed imperfect astrospheres. Nuclear division took place regularly but the cell body remained undivided so that so-called "syncytia" with as many as 64 nuclei arose. The same result may be produced by placing the eggs in hypertonic sea water (Loeb 1892, 1895[2]); if they are again placed in normal sea water cell division may be subsequently induced (Morgan 1894[1], Loeb 1895[2]).

In impregnated and non-impregnated eggs of the annelid *Chaetopterus pergamentaceus* which were exposed to certain mixtures of sea water with potassium chloride, Lillie observed the occurrence of "apparent" cell divisions which took place either without nuclear division or with a simple amitotic division in which no centrosomes were active. These divisions again disappeared but nevertheless a certain differentiation of the plasm is said to have taken place; the yolk withdrew in a denser mass into the interior, the peripheral protoplasm was vacuolated and became surrounded with cilia. This differentiation produced without cleavage presents a complete analogy with the endoderm, rich in yolk, and the ciliated ectoderm of the trochophore larva.

From all these observations and experiments the following conclusion may be drawn:

There is a normal association between division of the centrosome, formation of centrospheres, astrospheres, division of the nucleus and the yolk (cell body) and perhaps also progressive differentiation of the cleavage cells; but we must not conceive of this series in such a way as to suppose that one member is the determining cause of the next, but rather that all the processes are called forth in succession by a common cause, and then provide jointly for the typical development.

CHAPTER V.

MITOTIC CELL DIVISION.

(b) *Radiation of the plasm.*

FROM the independent occurrence of the several phenomena which together bring about the normal mitotic division of the nucleus and cell, namely, a divisible centrosome, radiate figures (astrospheres), nuclear spindles, divisible chromosomes, and segmenting cell bodies, we have inferred the existence of a single and more remote cause of mitotic division. Formerly this cause was considered to lie in the pre-existing structure of the cell; a network of fibres was supposed to be present even in the resting cell, each single thread of which was inserted on the one hand in the centrosome, on the other in the periphery of the cell (cytastrospheres), or in the chromosomes of the nucleus (central spindles); these were drawn together by a force proceeding from the centrosome, and accomplished the division of the chromosomes and cell body by mechanical means. Heidenhain [V, 1] constructed models to illustrate cell division according to this theory.

In his first model a series of rubber bands (the "rays") are fixed at equal intervals on the periphery of a circular disc (cell periphery). The bands proceeding from each half of the periphery are fastened at their other ends to a pair of rings which lie almost in the centre of the circle, and are at first fastened together. This is the aster stage in which the centrosome has divided into two parts (two rings). If the connexion between the rings is cut, they are drawn apart by the (centrifugal) tension of the rubber bands attached to each, since this tension is no longer counterbalanced by that of the other side, and they now assume a "diaster position." If a flexible circular band is employed instead of the circular disc the constriction of the cell may also be simulated [V, 2]. In addition to the difficulty of

applying the principle of these models of two dimensions, to those of three, the weakness of the theory lies in the behaviour of the nuclear spindle. This would arise between the centrosomes by the extension of strands of plasm. But the inner fibres of the spindle are not in a state of tension, on the contrary, they often exhibit a winding course and appear to grow without any appreciable diminution of their cross section. It is only the mantle fibres in the spindle which at first exhibit tension; these connect centrosomes and chromosomes and conduct the chromosomes, after division, to the diasters. Whether this must be regarded as due to contraction remains doubtful, especially since, in the cleavage of echinoderm eggs, for example, the chromosomes move into the centre of the diasters, a process which, as Wilson expresses it, "demands a contraction of the fibres almost to the vanishing point."

The mitotic forms have often been compared (Fol, Ziegler, Gallardo) to lines of force in an electric or magnetic field [V, 3—5], but this has not furnished any deeper analogy; in contrast to the electric and magnetic figures with dissimilar poles we are dealing in the case of mitosis with similar poles; this may be recognised from the migration of the chromosomes in opposite directions, and still more clearly from the association of several poles to form figures which do not in any way disturb the spindle and aster figures. The crossing of the rays has also been urged as an objection to this comparison.

A third theory has been propounded by Bütschli in connexion with his hypothesis that protoplasm universally possesses an alveolar structure, and this has been further developed by Rhumbler. Bütschli starts from the fact that the centrosomes and spheres are observed to increase in volume during mitosis, and he ascribes this increase to a withdrawal of water from the walls of the alveoli (the firmer "hyaloplasm"); this would establish a current in the structure directed towards the centrosome, and the structure would then arrange itself in a series of alveoli in accordance with the direction of the current. The model which Bütschli devised did not furnish a happy illustration. It consisted of a solution of gelatine, precipitated in alveolar form by means of chromic acid, and containing hot air-bubbles which afterwards contracted as they grew cold. The bubbles did indeed produce astrospheres all around them of the appearance and actual size of mitoses, and thus proved the possibility of creating a current in a colloidal semifluid substance; but on

account of the contraction of the air-bubbles in contrast to the increase of the centrosomes and their sphere, the experiment was no longer in accord with the observation which formed the basis of the theory.

Rhumbler, by supposing that the hyaloplasm became denser while the alveoli and their walls diminished, sought to demonstrate the existence of a tractive force exerted on the more remote hyaloplasm which is richer in water; this would produce the flow in the direction of the centrosome; Gurwitsch disputes the possibility of obtaining this effect in a fluid. In addition the assumption of a withdrawal of water from the walls of the alveoli is quite arbitrary. "If on the other hand we suppose that the centrosome swells at the expense of the enchylemma (cell sap) the walls of the honeycomb must naturally contract in a corresponding degree, with a decrease in the volume of the alveolar structure, and the effect of a current is produced; this takes place in a manner which completely resembles the process in the gelatine (Bütschli). The swelling up of the centrosome, which as before presents no radiation in its structure, at the expense of the surrounding sphere which is becoming radiate, is a fact established in a great number of cases."

If we disregard the "alveolar structure" of the protoplasm, which is not generally established, we may accept the accumulation of water in the centrosomes as the cause which determines mitosis. This agrees well with the explanation given of the determinant of the first mitosis, namely, the impregnation or other fertilisation with which development commences. Here the withdrawal of water from the egg plasm is recorded in the swelling of the spermatozoon which enters with its centrosome, or as the case may be, in the moisture extracting nature of the artificial means of fertilisation. The withdrawal of water, or rather of liquid, from the enchylemma during mitosis appears, as many histological pictures show, to take place from the cell body, especially in its periphery and from the chromatic nuclear mass, while the achromatic nuclear mass swells up, furnishes the spindle formation and appears to be surrounded by a large quantity of cell sap.

The withdrawal of water from the cell body reveals itself in the darker colouring of this part and the appearance of dense granules; the withdrawal of water from the chromatic mass, according to Haecker, causes the chromosomes to become clearly marked; these appear to present the same order in the resting state as in the

last stage of mitosis. This is supported by Boveri's observation that in the case of the egg of *Ascaris* they reappear in the same positions in the next stage of mitosis. Owing to their much swollen condition they cannot be distinguished as regards their power of refraction from the rest of the nuclear substance.

The concentration of cell sap during the mitoses often gives the cells at this time a tense appearance or increased "turgor."

Owing to the various processes of swelling we are able to refer the following partial phenomena of mitosis to a common cause [V, 7—12].

1. Increase in the size of the centrosome (in the case of impregnation, of the sperm nucleus also).

2. Formation of a centrosphere with associated radiations; in the case of artificial fertilisation this takes place near the cell nucleus without the presence of a pre-formed "centrosome."

3. Disintegration of the nuclear membrane and growth (swelling) of the achromatic spindle.

4. Distinct appearance of the chromosomes.

The division of the centrosome, the chromosomes and the cell body have still to be explained.

As regards the longitudinal division of the chromosomes it must be pointed out that their division (reduplication) has often taken place before their separation, indeed often before they become arranged in an "equatorial plate" at right angles to the nuclear spindle. It thus occurs almost (or precisely?) at the time when the chromosomes become clearly marked. It may therefore be permissible to imagine a longitudinal cleavage of the chromosomes occurring simultaneously with the outflow which takes place from them, since the solid matter thus separated, the chromosome-plasm, possesses less cohesion in a transverse direction than in its previous state when it contained more water. This solid matter is now exposed to the streaming movement of the enchylemma which takes place towards the centrospheres, as is shown by the experiments of Fischel who coloured the living egg of the echinoderm with neutral reds; the dividing halves are moved apart, and since they stand in contact with the mantle fibres of the spindle are thrust towards opposite poles.

With regard to the cleavage of the cell body, alterations of the surface tension come into question which are conditioned by the altered distribution of its liquid constituents. In the case of cteno-

phores Ziegler observed the advance of a denser layer of exoplasm into the first furrow which appears at the nuclear pole. We may also recall the fact that after fertilisation the egg is in a remarkably plastic condition, so that in the case of the sea urchin, for instance, it can be drawn out into long threads (Morgan, Driesch). As the chromosomes move apart the formation which was previously monocentric becomes dicentric, and around each new centre a special layer is defined by means of the surface tension. The moving apart of the centrosomes would be explained by the swelling up or some other mode of growth of the central spindle, and the cleavage of the centrosomes would be referred to a process similar to that which takes place in the chromosomes; this is naturally suggested by the development of the chromosomes from the nuclear substance in the case of *Infusoria*, as was demonstrated by R. Hertwig, and by their other relations to the nuclear mass. Here we may recall the intranuclear position of centrosomes destined to become those of the egg and the manner in which they are developed anew in the case of artificial parthenogenetic fertilisation.

As a provisional solution of the difficult problem of mitotic cell division we thus obtain the following statement:

The common cause of the mitotic phenomena lies in a localised secretion ("condensation") of a more liquid substance, the enchylemma, and in the transformation, caused by the redistribution of liquid, of a monocentric system of surface tension into a dicentric system.

CHAPTER VI.

ARRANGEMENT OF THE CLEAVAGE CELLS.

ACCORDING to Zur Strassen the centrosome takes up its position, at the termination of a mitosis, beneath the centre of the free surface of the cell, and according to Hertwig, the nuclear spindle lies in the direction of the longest axis of the cell. Hence the spindle of the second nuclear division, and the second furrow which stands perpendicular to it, may assume one of two positions.

The centrosome either divides in an equatorial direction and the nuclear spindle then lies in the equator, parallel to the first furrow, in which case the second furrow must fall in the meridian perpendicular to the first furrow; or the centrosome divides in this very meridian, and the nuclear spindle becomes parallel to the first furrow, but lies in the meridian which is perpendicular to it: then the second furrow must fall in the equator.

In the case of the frog and echinoderm the former of these two alternatives is realised. The determining cause may without prejudice be sought in the presence of the axial structure [IV, 16]. Only when the division takes place in the line of the equator do the parts formed by the cleavage of the centrosome move in portions of the egg which are of homologous structure. If frogs' eggs (Born 1894) are pressed between vertical sheets of glass, the nuclear spindles of the second furrow place themselves, in accordance with Hertwig's rule, in the longest diameter imposed on the blastomeres by the pressure, i.e. parallel to the plates exerting the pressure, and the second furrow is now an equatorial furrow [IV, 17]. Since, however, the masses of yolk which belong to the upper and lower halves of the egg are unequal the cleavage is also unequal, two smaller blastomeres being formed at the animal pole.

This mode of cleavage corresponds to that produced normally by the third furrow, and from this we may conclude that it is deter-

mined by the distribution of the plasm. This accords very well with the further developed cases of partial segmentation in eggs which contain an even greater abundance of yolk. That we are not dealing with a premature appearance of the third furrow, or with a complete or temporary omission of the second, is shown by the further course of the cleavage in the eggs under pressure. The third furrow of eggs pressed between vertical plates is parallel to the first furrow (again in accordance with Hertwig's rule), that is, it is neither a normal fourth nor a previously omitted second furrow. It rather corresponds to a third furrow, such as also arises, according to Hertwig's rule, in eggs pressed between horizontal sheets of glass, the second furrow having run quite (or almost) meridionally, as in normal cleavage [IV, 18].

If echinoderm eggs are subjected to pressure, in particular by means of Ziegler's aspirating-compressorium, they may produce a plate lying in one plane and consisting of 32 or more cells, owing to the constant placing of the nuclear spindles in a direction parallel to the compressing surfaces (Driesch 1892, O. Hertwig).

Pressure, the action of cold, or etherisation at the time when the first furrow begins to cut through the cell produce a suppression of the course of this furrow (Chabry, Boveri 1897, Wilson 1901[2], Teichmann 1903). The radiations disappear or do not occur at all, and the egg again assumes a spherical form. Nevertheless the nuclear division is accomplished so that a cell with two nuclei is now present. The second furrow may then appear normally although the first is not made good; Loeb (1895[3]) enclosed echinoderm eggs in the two- or four-cell stage in a moist chamber from which he expelled the oxygen by means of a current of hydrogen. The absence of oxygen causes the cells to absorb water and their volume increases so that the interior of the membrane is completely filled by the protoplasm of the cleavage spheres. The cell walls then disappear and the egg looks as though it were unsegmented. If oxygen is readmitted, and the eggs have not remained too long without it, they again segment. In many cases, but by no means invariably, the old furrows reappear.

Lack of oxygen has a liquidising effect on the eggs of fishes (*Ctenolabrus*) according to Loeb, as well as on *Infusoria* (Budgett) and on streaming plasm (Kühne).

Loeb brought forward the theory, well worthy of consideration, that the cell nucleus acts as an oxidising conductor in intracellular respiration, just as our red blood corpuscles serve to conduct oxygen.

Lillie, by means of microchemical methods first employed by Ehrlich for the oxidation channels in the tissues, found that the maximum of oxidation actually lies in the immediate proximity of the cell nucleus. According to Macallum the chromatin contains iron which, as is well known, plays the chief part in conducting oxygen in the case of the red corpuscles; and the work of Spitzer has shown that the oxidation ferments obtained in various extracts made from tissues belong to the group of nucleo-proteids, i.e. to the typical nuclear substances. If we may regard the nucleus as the centre of oxidation (or chemo-centre in the general sense) we have advanced another step in the explanation of cleavage. Factors which produce an acceleration of the oxidation processes (catalysers) will first cause more rapid oxidation and withdrawal of water, which will be followed by a more rapid exhaustion of the oxygen stored up in the nucleus: a lack of oxygen and consequent "liquidisation" will then occur in the cell. Then follow the processes of nuclear division and cell division connected with this condition, which transform a monocentric into a dicentric system. The chromosomes then begin to store up oxygen again (to respire), and at the same time they swell and grow (assimilation). In this process oxygen is again consumed, but the relation between the oxygen supplied and that used becomes continually more unequal, owing to the disproportion between the increasing absorbent surface and of the assimilating nuclear mass which stand in the ratio of the second to the third power. A dearth of oxygen again occurs and in this way the process may be rhythmically repeated. Quite recently R. Hertwig has insisted on the constant relation between the cell body and the cell nucleus as a relationship of the nuclear plasm. The principle propounded by Driesch of a fixed "cell magnitude," even in the case of several embryos developing from one egg, requires restriction according to Boveri. This observer holds that the cell volume is always proportional to the number of chromosomes and thus the nuclear surface is regulated with regard to attaining the normal nuclear plasm relationship.

In this connexion it may be pointed out that the division of the simple globe of the egg into several spheres creates a more favourable, i.e. a larger surface for respiration or other means of absorption. If the egg grew without division the conditions would become continually more unfavourable, since the volume would increase in the ratio of the third, and the surface in that of the second power. Finally a limit would be imposed on its power of continued existence.

This accords well with the small size of the unicellular organisms (Protists) and of the eggs of the multicellular.

While the unicellular organisms completely separate between the divisions, the divisional products of the multicellular retain their continuity.

The cause of this does not lie only in the egg membranes, which indeed the embryo abandons later, but in the presence of cementing substances. Herbst (1899) placed echinoderm eggs which had been deprived of their membrane by means of agitation in salt water devoid of calcium, and found that their cleavage cells fell completely apart; he then demonstrated the same fact for the cells of the ciliated larvae of *Polymnia nebulosa*, the heads of the polyp *Tubularia mesembryanthemum*, the epithelial cells of the larvae of the sea-squirt *Ciona intestinalis* and so on.

If echinoderm eggs which have fallen apart into their separate cleavage spheres in sea water devoid of lime, are placed in water containing lime, the cleavage cells formed by later segmentation remain associated; and indeed such as still lie loosely together may again unite. As regards the action of the calcium Herbst points out that we need not assume that it forms any combination, since e.g. "fibrinogen" passes into fibrin when calcium is present, but calcium does not occur in the fibrin in combination.

On the other hand sodium has a tendency to produce a loosening of the cell connexion (echinoderm eggs, gills of young eels—Herbst). This antagonism between sodium and calcium may explain the fact that eggs of the fish *Fundulus* are able to develop in distilled water and in that containing sodium and calcium, but not in a pure solution of sodium chloride.

The rules hitherto obtained also hold for the further cleavage. In general all the newly developed blastomeres divide again at the same time so that as the two-cell stage is followed by the four-cell stage, and then by the eight-cell stage, this again may be succeeded by sixteen-, thirty-two-, and sixty-four-cell stages. Or it may only be the descendants of a single region which divide simultaneously (Zur Strassen). In this way the egg is divided into a more or less spherical group of blastomeres. The cells produced by the division may be of different size and in general follow Balfour's rule that the greater the amount of reserve yolk contained in any part of the egg, the larger will be the cell produced by that part, probably, as we have mentioned above, because the yolk offers a greater resistance to the incision of the furrow.

The arrangement of the blastomeres takes place in accordance with the laws of minimal surfaces (Plateau) which hold for all drops of liquid.

Drops of liquid, which do not coalesce become grouped together in a definite manner until the sum of the surfaces under the given conditions is reduced to a minimum.

Roux suspended drops of oil in a mixture of alcohol and water so that they occupied almost the whole surface of a conically shaped wine glass; he then divided the drops, which ranged themselves together exactly in the same way as the first cleavage cells [VI, 1—4]. The drops might frequently be divided in different ways without altering the result, since the divided drops if displaced crept into the only possible position for producing the minimal area of surface contact.

By unequal divisions of the divided drops some of the cells may be forced away from the centre, and thus to some extent the development of a cleavage cavity may be simulated, as well as the thrusting of one of the cells into the interior, such as occurs in the stages which follow cleavage (gastrulation, formation of mesenchyme) [VI, 5].

The oil drops may also be divided horizontally so that rings of drops arise standing one over the other. The division is performed with a glass rod bent horizontally, in a fullbellied wine glass. Roux then showed by experiments on the frog's egg that displacements may actually occur in the arrangement of the blastomeres, such as are presented by the unequally divided oil drops. Individual blastomeres were pricked so that the escape of plasm ("extraovate") produced a diminution of their bulk. Displacements of the blastomeres then occurred generally leading to the arrangement which would be assumed by oil drops presenting similar differences of size. It must be admitted that this does not always occur. For cases of obstruction the incompletely fluid state of the substance must be held responsible.

Roux (1896) experimented on an "oil drop which, relatively to the cross section of the wine glass corresponded in size only to one half of the drop considered above as a whole egg; this he divided repeatedly in a radial direction from the interior to the exterior, then if there was no outward disturbance the drops remained lying against the periphery of the glass, and like it formed a semicircle [VI, 6], but if the oil was pure they were not flattened against one another, owing to the absence of the contrary pressure which the other half of the circle would exert. If however the oil was suffi-

ciently impure the surface tension sank, and the drops retained for some time the flattened sides and peripheral planes which arose during the division, and if a very thin glass rod was employed for the operation they lay close together. The flattening was more marked and more lasting if castor oil was employed, or better still a mixture of four parts of lamp oil with two to five parts of lard, according to the lower or higher temperature of the room. In the latter case, in addition to the probable diminution of the surface tension, the increase of viscosity must also be regarded as a factor which preserves the form produced by division."

It is therefore possible that the behaviour of a form when cut in half during cleavage is determined by its fluid or viscid consistency; in the former case it undergoes a rearrangement of the cells so that a nearly spherical form is again produced, in the latter the "half form" remains.

In addition to the purely mechanical changes of cell form and displacement of cells Roux (1894) described apparently active movements of the blastomeres of divided morulae and blastulae of frogs (*Rana fusca* and *R. esculenta*) under the name of "zytotropism" [VI, 7]; according to our nomenclature however these movements must be termed "zytotaxis." Roux isolated such cells with a needle and placed them in filtered white of egg, in a ½% solution of common salt, and in a mixture of these two fluids in equal parts. He then observed that when they no longer stood in any demonstrable connexion to one another they still performed movements towards each other; they began to become pointed at the ends turned towards each other and to approach by jerks until they were in contact. In enfeebled eggs, such for instance as those produced towards the close of the spawning season, these phenomena did not occur.

The cells having attained contact placed their greater planes together, a process which Roux terms "cell juncture" ("zytarme") [VI, 8]. In this they follow Plateau's law mentioned above, but with exceptions which may be referred either to disturbance owing to a different state of aggregation, or to the injury of cells.

"Many cells of the morula and blastula stage which, after isolation, have to a varying extent placed their plane surfaces in contact, separate again," a process which Roux (1896) terms "zytochorism" [VI, 11]. This separation takes place either by a rounding process in both cells or in one only, or by the appearance of gaping fissures at the plane of contact. Roux writes on the causes of this pheno-

menon: "the special significance of this voluntary separation of cells superficially united we do not yet know. I conjecture that it depends in many cases on changes in the qualities of the cells. Slow death is often connected, as I have observed in gastrulae and young embryos, with the disintegration of the epithelia accompanied by a rounding process in the cells, a phenomenon which I have termed 'Framboisia (finalis) minor' (cf. no. 3, vol. II, p. 1050). This quickly appears in embryos if salt solutions of too great strength are used as the medium....

"Further, Framboisia arises rapidly in the polar fields when the electric current is employed in the electrolytic field (cf. no. 4)."

Roux had previously made experiments on the effect of electric currents in determining the direction of furrows in the frog's egg and observed the following:

"When a current from platino-electrodes 1·7 cm. in breadth, was passed through a straight band of frog spawn 5—9 cm. in length, 2—2·5 cm. in breath and a single layer of eggs in height, each of the eggs, fertilised from one to three hours previously, presented within 15—30 seconds a distinct separation of the nearly spherical surface into three fields; these were parallel to one another, and consisted of two 'polar fields' with an altered surface turned towards the electrodes, and an 'equatorial zonal field' without any such change in the surface.

"This division of the surface generally begins with a lighter appearance in the region of the polar field and the development at first of a reticulate or punctuated lighter marking. Sometimes before any discolouration of the surface is perceptible, the two parallel circles appear on the lower hemisphere of the egg, which is light grey or often almost white, as two blackish lines, and thus accomplish the first visible separation into three sections.

"In the egg divided into two or more cells, as well as in the morula and even in the blastula, which is split into small cells, I observed that each cell in the surface of the egg became polarised independently" (Roux 1895[1], II, p. 591).

"If eggs with well rounded cells are slightly poisoned by brief immersion in water containing 5 °/₀ of a saturated aqueous solution of carbolic acid, they retain the round form of their cells, but the special polar fields which arose immediately on employing the current extend over the whole cell surface directly irradiated. Thus a uniform polar field, consisting however in the region of the upper hemisphere of

rounded projecting cells arises rapidly on both sides; between the two fields lies the general equator bounded by two continuous parallel lines" (Roux 1895[1], II, p. 596).

"Electric stimulus produces simple contraction. This might also occur at death and destroy the epithelial connexion if not yet sufficiently firm" (Roux 1896, p. 408).

Roux also observed the "creeping" of cells ("zytolisthesis") which in the case of separated blastomeres may take place so that they crowd together according to Plateau's law, in the same way as divided oil drops crowd round a glass. But as a rule the process produces extensive modifications, in consequence of the want of homogeneity in the different blastomeres, eventually calling forth the power of the cell substance to rearrange itself within the cell, sometimes in a manner determined by the position of the points of contact with adjacent cells; thus, for example, the pigmented cell substance will be arranged in the middle of the outer side of a complex.

And here we may mention Dreyer's (1892) studies on the formation of the skeleton which reveal the intimate connexion between the arrangement of the points at which the skeleton is separated and the drop form of the colloidal condition.

Our results with regard to the arrangement of the blastomeres in the first stages of cleavage, and in the morula and blastula, may be briefly expressed as follows:

The second and later furrows depend on a rhythmic recurrence of the conditions of supply which determined the first furrow. The entrance of oxygen determines the continuance of assimilation, the intensity of which varies with the change in the ratio of the absorbing surface to the assimilating volume and with the presence of antagonistic substances which may either hinder the complete separation of the blastomeres (calcium) or permit a certain degree of disintegration (sodium).

The arrangement of the blastomeres takes place according to Plateau's law of the minimal area of surface contact, which states the manner in which drops of liquid mass themselves together. Deviations from the law must be referred to the different consistency of different parts of the egg, and this is also responsible for the different size of the blastomeres formed (Balfour's law).

CHAPTER VII.

GASTRULATION.

THE process of cleavage terminates with the formation of the blastula; the individual cells still seem to resemble each other, although a closer examination shows that the cells of certain regions are characterised by differences of size and colour. At this stage many animal embryos such as echinoderms leave the egg envelope and on the outer surface of the cells develop cilia, which serve for propulsion. Nothing is known as to the causes of cilia formation. It is possible that a hint as to the direction which experiments should take is furnished by the observation made by A. Hinterberger and Reitmann (1904) that certain protists (*Bacillus pyocyaneus, Micrococcus agilis, Proteus*) develop flagellate and active or non-flagellate cespitose forms according to the greater or less amount of water contained in the fostering soil. The possibility of modifying the development of cilia in later stages will be discussed in dealing with the necessity of certain substances in the environment. The freely-swimming blastula grows by taking up water. This causes the cavity between the cells to increase in size, and the increased turgor gives the blastula a tense appearance. If however the medium does not possess the proper concentration compact and crumpled forms, the so-called "stereoblastulae" (Driesch, Herbst), arise.

If the blastulae of echinoderms are placed in solutions devoid of lime, the several cells become isolated, cilia are developed all around them, and it is impossible to determine which cells would have performed any given function if they had remained in contact. The blastula stage is the last in which the cells show no differentiation of structure or function, and it is also the last in which all the *Metazoa* can be included in a general description.

In the next stage the cleavage material becomes differentiated into two kinds of cells, the "ectodermal cells," which are generally

small and poor in yolk, and the larger, "endodermal cells," rich in yolk. The former furnish the outer layer which serves as the boundary between the organism and its environment; they thus perform the function of receiving stimuli from the outer world and also develop organs for propulsion in the medium in which they live (tufts and rings of cilia, etc.). The endodermal cells on the other hand serve chiefly to digest the nourishment which is either provided for the egg in the form of masses of yolk or must be conducted to the body from without by means of an oesophageal opening in the ectoderm. More favourable conditions are created for the absorbing surfaces by continued cell divisions, especially by invagination; for by this means the surface is increased in relation to the volume in need of nourishment to a much greater extent than by the previous division into cleavage spheres, and the thrusting apart of these spheres by a "blastocoel" containing fluid. As is well known, the complex of ectodermal cells is distinguished as the outer germ layer ("ectoderm") from the complex of endodermal cells or inner germ layer ("endoderm"). The separation of the embryo into ectoderm and endoderm takes place in a great variety of ways, and when we term all the different processes "gastrulation" we must remember that this term defines a homologous stage, namely that in which the first two germ layers or "primitive organs" are developed from the blastula, but that it does not imply analogous mechanical processes.

Before attempting to trace the connexion between one process of gastrulation and another we must first answer the question as to what forces cause the endodermal cells to reach the interior of the blastula. Even in the coeloblastula we find that the principles stated for the arrangement of compact cell groups according to Plateau's law no longer hold good. It would be natural to suppose that the blastocoel fluid which is secreted by the blastula walls into the interior is responsible for the maintenance of a pressure exerted in a centrifugal direction which would hold the cells at the surface in a densely crowded layer. But in abnormal cases open blastulae have been observed, by Morgan and Hazen for instance in *Amphioxus*, and a similar phenomenon was obtained by Driesch, who isolated the first blastomeres of echinoderm eggs; these produced a widely open half blastula composed of a single layer. From this and from his own observations in the case of *Ascaris*, Zur Strassen (1903[1]) draws the logical conclusion that an excess of pressure in the interior cannot be regarded as the

cause of the surface arrangement, since in these cases there is no difference of pressure within the blastocoel and without it. Zur Strassen shows further that the epithelial arrangement is only possible on the supposition of an "anisotropy" of the several cells. A plane epithelium will be produced if the individual cells, regarded as spherical, have formed an "attraction zone" along their equator which causes them to press against each other and become flattened, while the polar domes remain arched. A bent epithelium would be produced if the attraction spheres were displaced in the direction of one of the poles, since the flattened surfaces would then converge towards these poles so that the latter would occupy the inner side and those opposed to them the outer side of the arc. But even this supposition does not afford a sufficient explanation, since the same cells are able to produce extremely different epithelia according to their position. Zur Strassen therefore imagines the further complication, that each cell falls into parallel attraction zones in a series of layers. The effect of this is that although the cells are able to lie close together in a variety of ways by means of every two similar attraction spheres, the position of similar attraction spheres is analogous in all the cells at the same level, and thus the latter are held together in a single epithelial layer. Zur Strassen sees ground for the probability of this supposition in the turning of the centrosomes after the divisions, which, as we have mentioned above, he traced to the inequality of the plasm and its symmetrical structure with regard to the axis of the cells. The attraction zones must be regarded as equatorial to the cell axis, which is the line uniting the centres of the free outer and the basal inner surface of the cell with a resting nucleus.

The question as to what forces determine the attraction Zur Strassen leaves undecided.

The mechanism of the gastrulation process was made the subject of an analytical investigation by Rhumbler (1902), who constructed models made of strips of steel fastened together to illustrate the different reactions of mechanical systems on alteration of the pressure, bulk of the elements, etc. [VI, 16]. Rhumbler comes to the conclusion that a migration of endodermal cells into the interior of the blastula cannot be called forth by passive forces such as would be produced by pressure of the ectoderm, suction of the diminished blastocoel, etc., but that the endodermal cells possess a power of active movement which must probably be traced to chemotaxis in

the same way as was done some time ago for *Amoebae*, to which the migratory vegetative cells are often morphologically similar. The next change of form necessary Rhumbler explains by the fact that the surface tension decreases on the side of the vegetative cells turned towards the interior owing to some chemical difference set up in the blastocoel fluid, such as for instance its enrichment with the carbonic acid separated in gas assimilation, whereas on the outer surface a current of oxygen continually received from the surrounding medium prevents any such change. The decrease of the surface tension however may be followed by a welling forth of plasm (extension of pseudopodia), which, given miscibility, attracts material to itself from the blastocoel fluid. New differences of tension are thus set up, and so by a repetition of the process plasm continues to advance still further into the interior. The reason that the chemotactic action only affects the cells of the vegetative pole lies in the fact that this pole possesses from the beginning a different chemical composition, which finds expression in the different character of the blastomeres. In brief therefore we may say :

Blastulation and gastrulation depend on chemotactic effects started by processes of assimilation, which not only cause passive mechanical displacements, but also active migration of cells.

CHAPTER VIII.

MECHANISM OF THE DEVELOPMENT OF DIFFERENTIATION.

1. *Cnidaria*[1].

If we trace the history of the several blastomeres and their descendants the "cell-lineage," until differentiations appear of which the future organic significance can be recognised, we are able to state what parts of the embryo arise normally from the several regions or blastomeres of the egg. That which is normally developed in the future embryo from a blastomere in any given position in the egg we will term with Driesch the "prospective significance" of that blastomere. The question now arises whether any given organ can be formed only from a particular blastomere (or from a blastomere in a given position), or whether a change in the course of development, in particular a reduction of the egg material by the removal of its substance or of blastomeres, would still permit those organs to be formed which would normally owe their origin to the part of the egg removed. It appears in fact that a given blastomere or a given part of the egg is not only able to develop that which corresponds to its "prospective significance," but under certain conditions may assume the rôle of the other blastomeres or parts of the egg. Driesch terms everything which a blastomere is able to produce, even under altered circumstances, its "prospective potency."

The question as to how far the rudiments are predetermined in definite parts of the egg, or in what relation the future parts of the embryo stand to the cleavage planes has been termed the "problem of determination" (Korschelt and Heider).

A number of forms have been investigated with regard to the possibility of obtaining them from isolated blastomeres or pieces of egg: such for example are certain *Hydrozoa*, the Actinozoan *Renilla*,

[1] With regard to the *Porifera* see pp. 91–93.

and some of the ctenophores. We must first mention the earliest experiment in division of the egg made by Haeckel (1869) on the Siphonophore *Crystallodes*, in which, when embryos of the second day were cut into from two to four pieces, complete regeneration took place.

Martha Bunting separated the first two blastomeres of *Hydractinia* by shaking them or cutting them apart, and obtained perfect planulae of half the ordinary size. Whether the development of the endoderm corresponded to the normal multipolar delamination could not be determined with certainty.

Zoja (1894, 1895) summarised as follows the results of his experiments on the first stages of cleavage in *Liriope mucronata*, *Geryonia proboscidalis*, *Mitrocoma annae*, *Clytia flavescens*, and *Laodice cruciata* [VII, 1, 2]:

"The separation of the blastomeres in medusae was made with a very fine steel needle ground into the form of a lancet, at the moment when the cells were least in contact; the latter are not in any way altered by the process. The development of the separated blastomeres ($\frac{1}{2}$ and $\frac{1}{4}$ of an egg of *Liriope*, *Geryonia* and *Mitrocoma*, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ of an egg of *Clytia* and *Laodice*) resembles exactly in all its stages that of the intact egg. The cleavage cavity is always closed and central, and in no case are processes indicating regeneration to be seen during the development of the embryos which have arisen from isolated blastomeres. Finally a swimming larva is always formed, consisting of two kinds of tissue, and this cannot be distinguished in any way, except in its dimensions, from a larva developed from an intact egg.

"In *Clytia* halves and quarters of eggs attained a completely developed hydroid form, and in *Liriope* half an egg produced a small round medusa in which the four primary tentacles were arranged in the normal cross.

"From an eighth of an egg of *Liriope*, which yet contains material from both ectoderm and endoderm, a larva with two kinds of tissue is not developed.

"The number of cells in the larva developed from $\frac{1}{1}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ of an egg in the case of *Laodice* and *Clytia* appears to stand in the ratio of $1 : \frac{1}{2} : \frac{1}{4} : \frac{1}{8}$ when the endoderm first begins to form. This is not so in the case of *Liriope* where the larva from half an egg first forms an endoderm at the transition time from the 16- to the 32-cell stage ($\frac{16}{32}$—$\frac{32}{64}$), as is the case in the complete larva. This difference

between the various medusae is in all likelihood determined by the different dimensions of the cleavage cavity." This cavity is so small in the 16-cell stage of half an egg of *Liriope* that it would probably present a mechanical obstacle to the migration of the endoderm cells, and this would only be obviated by the growth of the cavity in the following stage.

O. Maas (1901[1]) has carried out further experiments on the comparatively large eggs of *Aegineta flavescens*, a form with which we have already become acquainted in discussing the structure of the egg [VII, 3—5].

These eggs occur in various sizes so that, as Maas observes, nature herself has already experimented as to the possibility of obtaining from a diminished mass correspondingly diminished medusae. Besides the variation in size he also observed two different methods of cleavage, either eight equally large, or four larger and four smaller blastomeres being formed. The furrows showed no relation to the plane of gravity or to the future major axis of the medusa.

"No cleavage cavity is formed, but even during earlier divisions first a few, and then an increasing number of cells reach the interior. These inner cells consist chiefly of the frothy endoplasm containing the yolk; the cells situated at the surface may at first contain both kinds of plasm, exoplasm and endoplasm, but during later divisions they continually lose some of the latter until at last a covering of cells consisting only of exoplasm is formed.

"These cells develop cilia, and the planula, first of spherical and then of a more oval form, swims freely in the water." The planula then becomes flattened in the form of a disc and develops gelatinous tissue, tentacles, umbrella and manubrium.

If eggs in the four- or eight-cell stage were cut into two parts the result was different according as these parts consisted of cells of equal or of different size. When Maas, for example, divided eggs in the eight-cell stage which had formed *equal* blastomeres, then the embryos, each consisting of four equal blastomeres, developed like whole eggs and became perfect but diminutive medusae, the blastomeres rapidly closing up to form a sphere. Four-fifths of the embryos developed.

The course of the experiment is somewhat different when made in a form containing unequal blastomeres, e.g. in the eight-cell stage [VII, 5], in such a way that the four small cleavage spheres are contained in one part, the four large spheres in the other.

The four small blastomeres at first continue to divide, but remain almost in one plane [VII, 5*a*]; no rounding to form a cell group occurs and the divisions soon cease. The cells frequently move completely apart and the whole of these part-formations invariably perish. The four larger blastomeres however divide vigorously and form a morula, but this possesses an irregular structure and its cells are of unequal size. In a number of cases development may continue as far as the ciliated planula and even until tentacles begin to appear [VII, 5*b*]; many abnormalities however occur, especially in the position of the tentacles with regard to the umbrella margin, and sometimes large lumpy blastomeres remain in the endoderm which is otherwise further divided and of epithelial structure. Much of this may be regulated later, but on the whole the result of raising embryos from very large blastomeres, although more favourable than that from small, which always perish, is yet bad in comparison with the first mentioned experiments with equal fragments; for scarcely a quarter of the products of isolation reach the larval stage.

The results are similar if we make the division in a form containing unequal blastomeres, so that each part contains two smaller and two larger blastomeres. In this case rounding, the formation of layers, and even later stages *may* be attained, but it is not the rule as in the case of the isolation of equal cells, and the products are considerably more irregular and distorted.

By means of repeatedly driving the cells in and out of a pipette with a broad mouth Maas also succeeded in drawing out the mass of cleavage cells until they formed a single row of cells like a thread alga [VII, 6]. These again closed up to form spheres, and continued to develop normally although the secondary arrangement of the cleavage spheres sometimes differed considerably from the primary. In all the experiments the distribution of the exoplasm and endoplasm depended upon the position of the cleavage spheres in the egg. The surfaces bordering on the outer world received a covering of ectoplasm, but not the boundary walls between two cells.

This redistribution of the substances determining the germ layers must certainly be the factor which renders possible the development of diminutive but perfect forms among the hydroid polyps. In the case of those eggs of *Aegineta*, in which unequal cleavage spheres occur, the comparative lack of endoplasm in the part containing the micromeres and the comparative lack of ectoplasm in that containing the macromeres determines the arrest of development.

The reason why a fragment consisting both of micromeres and macromeres should give comparatively unfavourable results is not so clear to me; perhaps there is some deficiency in the power of rearrangement possessed by the two substances, a supposition supported by the distortion and irregularity of the final products. Possibly also other unequal divisions are already present which are not yet visible to the eye of the observer.

In *Clytia flavidula* (= *Phialidium flavidulum*), Maas (1905) obtained perfect diminutive forms from fragments of planulae. All irregularities he refers to the fact that in the later stages the ectoplasm no longer surrounds the exposed surfaces freely and completely.

Charles Hargitt (1905) also obtained perfect and diminutive hydroid polyps from fragments of eggs of *Pennaria tiarella* in different stages of development.

With regard to the *Scyphozoa* we possess a few observations made by E. B. Wilson (1903[2]) on the sea-pen (*Renilla*) which belongs to the corals (*Anthozoa*) [VII, 7—9]. This observer cut up eggs in various stages, between the laying of the egg and cleavage, and obtained diminutive perfect forms [VII, 7], which developed with the same rapidity as normal eggs; the sum of the blastomeres contained in each part corresponded to the normal number of blastomeres in an egg. A peculiarity of *Renilla* is that a division of the nucleus into from three to five parts occurs normally before the cleavage of the cytoplasm, and then eight to sixteen blastomeres are produced simultaneously. If the eggs were cut up before the nuclear divisions had taken place, only one fragment, that containing the nucleus, was able to develop, and into those without a nucleus the spermatozoa appeared unable even to enter. In the later stages, when the nucleus is already divided, several diminutive embryos may develop from one egg, since each fragment may evidently contain a nucleus. Even a fourth part of the mass of the egg puts forth the first two buds at the same time as the intact egg and, like the latter, is evidently capable of giving rise to the Zoantharian colony [VII, 9]. Beyond this stage even normal embryos cannot be raised.

2. *Ctenophora.*

The behaviour of the eggs of the *Ctenophora* presents a strong contrast to that of the hydroid polyps.

Concordant results have been obtained in this group by Chun (1892), Driesch and Morgan (1895), Fischel (1897, 1898) and Ziegler (1898).

Normal development, independent of the force of gravity, takes the following course [VIII, 1]. The egg possesses a cortical layer (ectoplasm) and an endoplasm formed of transparent spheres of yolk; the nucleus lies close to the periphery. The first furrow begins to appear on the side near the nucleus and divides the egg into two equal parts. The second furrow is perpendicular to the first, so that the blastomeres of the four-cell stage are of equal size. The third furrows divide the four blastomeres into unequal parts, each blastomere first appearing to constrict off a smaller cell at the nuclear pole. Eventually the furrow draws further away from the nucleus owing to the overflow of yolk material from the opposite part of the egg, until finally somewhat smaller cells are constricted off at the proto-vegetative pole.

The eight blastomeres then lie side by side in two somewhat bent parallel series, the smaller cells forming the outer flanks. Two planes of symmetry are present:

1. The plane which separates the two parallel series, the *T*- (funnel or tentacle) plane, and

2. The plane perpendicular to the *T*-plane which divides both series into one big and one smaller blastomere, and is known as the *S*- (stomach) plane.

That the stomach plane corresponds to the first and the tentacle plane to the second furrow, was shown by Chun's isolation experiments and confirmed by observations of Ziegler and Fischel.

The eight-cell stage is followed by the formation of very small cells, or micromeres, the furrow at first approaching the prot-animal pole, but the micromere is finally constricted off at the vegetative pole. New micromeres arise by cleavage on the part of the macromeres and by divisions, often unequal, of the micromeres already present. The macromeres also experience a temporary increase, but divide at a much slower rate and become surrounded by the rapidly increasing micromeres; at the same time four bands of very small ecto-

dermal cells become differentiated in the diagonal planes of the embryo and these produce the pairs of "ribs" of the ctenophore larva. The ribs converge towards the micromere pole where an apical organ is formed destined to discharge sensory functions. After the separation of the mesoderm the macromeres, which develop only into endodermal cells, become completely surrounded. Opposite the apical pole an ectodermal oesophagus becomes invaginated at the pole which was originally the animal pole and divides the endoderm into two parts, so that it attains to great dimensions in one plane. This plane is the "stomach plane," since the oesophagus is also described as a stomach. The stomach afterwards communicates with a central cavity or funnel lying transversely to it, and from this the funnel plane takes its name. Since the later stages of many forms, known as the "tentaculate ctenophores" (*Eucharis*, *Bolina* and many others) exhibit two long (ectodermal) tentacles in the same plane towards the apical pole, this plane is also termed the "tentacular plane." The endoderm develops four "pouches" separated by the stomach and funnel plane.

Fischel (1903) cut sections of the fertilised but unsegmented egg of *Beroë ovata* and was able to show that four zones are present which by self differentiation develop severally mesoderm or ribs, ectoderm, or endoderm.

If the first blastomeres of the ctenophore egg are isolated each of the two cleavage cells produces a ctenophore which possesses only four ciliated bands, that is, half the normal number. Such larvae were first observed by Chun in the tentaculate forms *Eucharis* and *Bolina*. After storms they occur in the natural state, and Chun therefore supposed that they arose from eggs which had been mechanically agitated, a conjecture which experiments in isolation proved to be correct. The blastomeres of the atentaculate *Beroë ovata*, the eggs of which are 1·2 mm. in diameter, may be very easily separated with a sharp needle (Driesch and Morgan 1896, Ziegler 1898) [VIII, 2].

By pressing in the egg membrane between the blastomeres several embryos may be caused to develop, according to Fischel, within the intact covering [VIII, 4].

Even after further division individual blastomeres or groups of blastomeres may be isolated and a corresponding number of embryos obtained from one egg.

In every case the result as regards the development of the several

organs is the same; each reduced embryo possesses the number of ribs which would normally develop from that portion of the egg to which it owes its origin, while each blastomere cannot form more micromeres than it would have formed in the intact egg; from each original blastomere a row of cilia is produced as in normal development. That the formation of vibratile plates is unconditionally determined by the individual micromeres may be shown by displacing the blastomeres either by pressure in the aspirating compressorium or by squeezing up folds in the eggs and so confining the blastomeres. Wherever one of the original micromeres is present a row of cilia appears [VIII, 3]. If in the later stages when the original micromere has already divided, displacements or separations are made, the ciliated plates of a single rib may appear in different places, from which we must conclude that there are special micromeres for the development of each ciliated plate.

When we examine closely a larva produced by isolating the two cells of the two-cell stage, that is a larva with four ciliated bands, we perceive that one side is more convex than the other, which is that originally in contact with the other blastomere. The pairs of ciliated bands do not occur at perfectly regular intervals on the cell periphery, but exhibit a greater interval on the flat side. The regulation of the outer form is not perfect. Neither does half a larva represent a true "half form." The ectoderm has surrounded not only the convex but also the flatter side; the apical organ though regular in form possesses only half the normal mass; the so-called stomach arises on the margin of the flatter side opposite to the apical organ (i.e. in the place which would be normal in the intact egg), but then, instead of moving straight upwards, takes an oblique course towards the apical pole. In this way one of the endodermal pouches (of which there are but two in place of the normal four) cuts off a small space adjoining the flatter side, which has been described by Driesch and Morgan as a "third smaller endodermal pouch." The complete envelopment of the endoderm, the regular form of the apical organ, the alimentary canal rising closed into the interior instead of lying open on the intersecting plane, and the "third" endodermal pouch thus produced—all these developments in excess of the normal may be referred straightway to the inorganic factor of surface tension which will not permit the existence of open half formations. Tactile irritation of the various cell groups among each other may also be a factor, as for instance in the envelopment of the egg by the ecto-

dermal cells, which takes place so long as the micromeres can creep along endodermal cells lying beneath them, or in the ingrowth of the alimentary canal which everywhere strives to come into contact with the endoderm.

Larvae developed from small fragments of the blastomere material behave in a precisely similar manner to those developed from half the material.

By symmetrical displacement of the micromeres regular double formations can also be obtained in a single egg (Fischel 1898). If for example the blastomeres of the 16-cell stage are pressed towards the stomach plane with a fine pincers or the back of a knife, so that the macromeres on both sides of this plane turn the longer instead of the shorter axis towards each other, thus moving the micromeres still further apart, then each of the separated groups of micromeres forms an apical pole with four bands of ciliated plates, while a single intestine rises from the middle of the opposite pole.

Making a general survey of the experiments on ctenophore eggs we must define their development as a process of self differentiation of the several blastomeres.

A fragment of a blastomere only produces the differentiations corresponding to itself; regulation during larval development only extends to imperfect rounding and closing up, not to the completion of organs as regards mass or number.

Chun (1892) observed sexual maturity in the half larvae (*Eucharis*, *Bolina*). The same observer had described under the title "dissogeny" the remarkable fact that the ctenophores become sexually mature for the first time as larvae and for the second time after the completion of their metamorphosis.

After the metamorphosis Chun's half larvae began to show regeneration of the missing half [VIII, 5]. *Beroë* has not yet been reared as far as these stages. Divided adults of this species show no trace of regeneration, although they may live for a long time (Eimer).

3. *Echinodermata.*

We have already become acquainted with the eggs of echinoderms, and in particular of sea urchins, as types of regular cleavage [I, 2—9; IX, 1—6]. A holoblastic cleavage is followed by a gastrula stage with typical invagination. During the invagination of the endoderm separate cells become detached from its blind end, travel as mesen-

chyme to definite places and assume a bilaterally symmetrical arrangement. A tuft of cilia is formed at the animal pole. Two coelomic sacs become separated off from the endodermal canal; the blastopore becomes the anus, the intestine is separated into three parts lying one behind the other, and breaks through to form a mouth on one side below the tuft of cilia; around this mouth a flatly depressed square "mouthfield" is marked out by the development of cilia.

Meanwhile two groups of mesoderm cells (limeformers) have begun to develop a calcareous skeleton. This consists of long needles and (in the case of the sea urchin) grows outwards in four arm-like protuberances, each with a rod, which form at the corners of the mouthfield. The further development takes a different course in the various classes of echinoderms, for each class at first produces typical swimming larvae (sea urchin—pluteus, star-fish—bipinnaria, sea cucumber—auricularia and so on) which afterwards undergo a complicated metamorphosis and become transformed into creeping adult forms. We need not enter into a detailed description of these differentiations since they have not been made the subject of experimental investigations.

The first stages, however, have been rendered familiar by a very thorough experimental study. Driesch placed the eggs of *Echinus* half-an-hour after impregnation in sea water diluted 30% (1893, 1896); this caused the first two blastomeres to become loosened and larvae arose elongated in a direction perpendicular to the first furrow. The further development showed that this plane of extension generally coincides with the median plane of symmetry of the pluteus, that is the first furrow is perpendicular to this plane, separating an anterior from a posterior blastomere.

It appears exceptional for the first furrow to run equatorially and to separate the vegetative and animal blastomere. Coincidence of the first furrow and the median plane has never been observed. The first cleavage cells are perfectly capable of representing each other, at least in those eggs with typical cleavage.

If one of the first cleavage cells is isolated by mechanical agitation (*Echinus microtuberculatus*—Driesch 1891, *Sphaerechinus*—Driesch 1891, Morgan 1895[3], *Strongylocentrotus lividus*—Zoja 1895) it segments as a half form, but produces an entire individual (gastrula, pluteus) with half the number of cells [IX, 9]. Separate cells of the four-blastomere stage behave in a similar manner; while the number

of cells is proportionately reduced, the normal number of organs for the intact egg is developed though consisting of fewer cells [IX, 8]. After the appearance of the third typically equatorial furrow which completes the separation of the animal and vegetative material, isolated blastomeres will give rise to a complete number of animal organs alone, or of vegetative organs alone [IX, 7].

In the 16-cell stage the normal larva consists of four micromeres and four macromeres in the vegetative half of the egg and eight mesomeres in the animal half. In gastrulation the micromeres furnish the mesenchyme, the mesomeres the ectoderm, the macromeres the endoderm. If, in the 16-cell stage the four micromeres and the four macromeres, the vegetative layer proper, are separated from the eight mesomeres, the larvae produced from the latter are distinguished by their extremely long cilia and the absence of the intestine from those of the vegetative layer which lacks the typical lower differentiations of the ectoderm [IX, 1*a*, *c*]. It is a testimony to the value of the experimental method that Driesch was able, on the results obtained by experiments in separation, to assign their true significance to the poles, hitherto wrongly determined; this result afterwards received brilliant confirmation in Boveri's rediscovery (1901) of the orange red pigment ring in *Strongylocentrotus lividus*, which could now be directly traced to the poles of the oocyte. The development of the intestine always begins in the "most vegetative" part of a divided larva. While individual blastomeres of the 16-cell stage on isolation are no longer capable of producing an embryo, fragments of later stages, if they contain a greater mass, have this power (Morgan 1901).

Blastulae when cut up (*Sphaerechinus*, *Asterias*—Driesch 1895[2]) or shaken to pieces (Morgan 1895[4]) form small but perfect gastrulae; gastrulae when cut up (Driesch 1895) may form diminutive copies of the intact plutei of *Sphaerechinus* and bipennariae of *Asterias*.

"If the mesenchyme cells of the Echinid blastula, after their segmentation from the endoderm, are shaken into entirely abnormal positions in the larva, these cells, all or almost all, migrate to places which would be normal for a later stage of the ontogeny" (Driesch 1896[2]). This results in quite normal development. We may conclude that even in normal migration the mesenchyme cells reach their ultimate position by a tactile irritation of the adjacent cells, perhaps by "chemotaxis" (Herbst).

Eggs which are shaken up after the mesenchyme has been com-

pletely formed (*Echinus*—Morgan 1895[4]) develop abnormal larval forms owing to the lack of skeleton; the causal connexion in this case will be discussed in dealing with the dependence of development on external factors.

Embryos which are cut up after they have completely developed their mesenchyme and are beginning to form an intestine (*Sphaerechinus granularis, Echinus microtuberculatus, Asterias glacialis*) are able when they retain neither intestinal nor mesenchyme cells, to close the wound, regulate their form and develop ectodermal organs in the typical manner, but are not able to form anew the organs which have been removed (Driesch 1895[2]) [IX, 2*a*, *c*].

If archentera are isolated they do not develop further (Morgan 1895[4]): larvae which have lost the abnormally extruded archenteron cannot redevelop it (Driesch 1892). The secondary archenteron cannot again form the coelom sacs after they have once been removed (Driesch 1895[2]); if for example in operations on *Sphaerechinus* gastrulae one of the two triradiate spicules which represent the rudimentary skeleton was removed, then the skeletons of the plutei developed from these gastrulae were unilaterally developed [IX, 4*b*]. The mechanism of skeleton formation and its development *de novo* will be discussed again on a later page.

The power of the blastomeres to represent each other in the earlier stages, which afterwards decreases in proportion as the differentiation proceeds, may be demonstrated by technical methods other than cutting or shaking them apart. Loeb obtained his results by diminishing the concentration of the medium and this was really the earliest method employed in the case of sea urchins.

An addition of 20—25 °/₀ of fresh water does not produce any important change in the development of eggs of *Echinus* or *Sphaerechinus* (Herbst 1897): a still greater dilution however (100 °/₀ *Arbacia*) causes osmotic disruption of the embryo (Loeb 1894).

If such a division is made in the two-cell stage, the plane of disruption stands in no definite relation to the first cleavage plane. Nevertheless extra- and intraovates develop like complete eggs [IX, 11]. From Boveri's observations on *Strongylocentrotus* however we must conclude that the products of division have received something from all the zones of the egg in order to develop completely and that it is therefore not a case of complete "isotropy" of the egg.

Up to the 32-cell stage extra- and intraovates may be induced to form by means of diluted sea water; they afterwards develop into

complete blastulae and those containing, as a minimum, one eighth of the mass of the egg may even develop into plutei. "If eggs which have just entered the blastula stage are placed in diluted sea water, the membrane bursts and a prolapse of the blastula occurs." On the other hand Loeb was able to convince himself that the wall of the blastula never bursts. "If the egg was replaced in normal sea water the blastula again assumed its spherical form." From these blastulae simple embryos were always developed.

Herbst (1897) obtained several larvae from one egg by the addition of 6 grams of potassium bromide (KBr) to 1000 c.c. of sea water, and also by using potassium iodide (KI), sodium bromide (NaBr), nitrate of soda ($NaNO_3$). In two cultures consisting of four parts sea water and one part 3% potassium chloride (KCl) there were a number of cases in which two blastulae occurred together in one egg membrane, either connected or separated; it is probable that this was also due to osmotic separation.

If pressure is exerted on segmenting eggs by means of a cover glass (which should be supported on a hog's bristle) then the flattened 8- and 16-cell stages (sea urchin—Driesch 1892) immediately re-assume their spherical form when the pressure is relaxed, provided that the membrane remains uninjured, but the cells and spindles occupy different positions. If the egg membrane bursts a second layer is not formed until the next division.

Normal plutei may arise in all these cases. If eggs (*Arbacia pustulata*—Morgan 1894) are subjected to pressure in the four-cell stage, the first and second furrows are vertical; the third furrow is also vertical (normal); if the eggs are rotated 90° and pressure again exerted, micromeres are formed laterally.

If *Echinus microtuberculatus* in the eight-cell stage is shaken fairly hard for about a minute all the cleavage cells lie in the same plane (Driesch 1896[3]); nevertheless normal plutei may result. It is necessary, however, in all experiments in displacement that the micromeres should finally come together again if only one vegetative pole is to be formed[1].

It is not only by experiments on separation and displacement in echinoderm eggs that we have become aware of their power to produce a uniform whole by regulation. In many cases it has also

[1] Otherwise there will be as many vegetative poles as there are separate micromere groups.

been possible to obtain from two eggs, by means of coalescence, embryos which are uniform but of double the usual size, because consisting of double the number of cells [IX, 13]. Morgan deprived (1895[1]) eggs of *Sphaerechinus* of their membrane by shaking them two minutes after impregnation, and then let them fall a foot into a dish of sea water, where they adhered together at the bottom. The blastula stage was followed by coalescence; generally two intestinal ingrowths took place, but one might overtake the other in development and then the whole (two-fold) wall grew around the first of these as a centre and formed a single larva. One skeleton extends through the whole length of such a larva; the rudiment of a second skeleton may also be present.

Driesch (1900[1]) added sperm to the eggs of *Echinus* or *Sphaerechinus* and, after 3—5 minutes, shook them moderately hard for thirty times in a small glass. Ten minutes later he placed them in alkaline sea water free from lime, to which ½ % sodium hydrate had been added in the proportion of six drops to 20 cms. (according to Herbst). Twenty eggs in every 1000 coalesced. Only those which coalesced early gave simple forms; the others produced double growths variously deformed. Doubtless the direction of the egg axes with regard to one another is also a determining factor, as well as the time at which coalescence takes place; for a completely uniform coalescence could only occur if the egg axes were parallel to one another (Boveri).

Finally Garbowski (1904[1]), by combining different methods, succeeded in transplanting blastomeres. Fragments of divided eggs (*Psammechinus miliaris*) were coloured intravitally with neutral red, which does not injure the larvae [IX, 12]. These and other fragments, either uncoloured or coloured with different reagents, methyl blue or phenyl brown, were caused to coalesce either by placing them under strong hydraulic pressure in a long burette or by directly pressing the fragments together with glass-headed pins.

Embryos such as these composed of the blastomeres of several individuals were able to develop uniform plutei, by means of various regulation processes, even when the blastomeres were taken from eggs in different stages. When the micromeres were absent, mesenchyme was still formed; from this it must be inferred that the other vegetative cells (macromeres in *Strongylocentrotus*) are also able to form mesenchyme.

4. Worms (*Vermes*), and kingcrab (*Limulus*).

Among the forms generally classed together as "worms" the following have so far been investigated with regard to the localisation problem; these are: the nematode *Ascaris megalocephala*, the nemertine *Cerebratulus*, and the annelids *Lanice* and *Chaetopterus*.

(*a*) Round worms (*Nematoda*).

The normal relations of the several blastomeres of *Ascaris* to the later organs and portions of the body are described by Zur Strassen in the following account, based upon his own results and those of Boveri [XI, 1, 6].

"The egg of *Ascaris megalocephala* is divided by the first cleavage into cells lying one above the other, which differ fundamentally both in their prospective significance and their mode of development. From the smaller cell which lies beneath are produced the greater number of the organs, the sexual system, the mesoderm, the whole of the digestive tract and a part of the body wall. This lower cell group, which is confined to the ventral side and the posterior end of the animal, divides with a very irregular rhythm, but all its descendants are sharply characterised, even in the later cleavage stages, as regards their mutual position and the nature of their nuclei. On the other hand the larger cell lying above furnishes a single structure, the "ectoderm," which develops at a uniform rate. In accordance with its general position we are accustomed to describe as ectoderm the developing cell-plate, finally bent in the form of a hood, which occupies by far the greater part of the embryo, viz. the whole of the dorsal and lateral parts. The ventral and posterior parts are connected with the products of the lower cleavage sphere and partly envelop them.

"The most valuable part of the work (of Boveri), however, consists in the beautiful discovery that the separation of the germ cells and soma which is theoretically required by Weismann is actually realised in the case of *Ascaris*. After a very few cell-generations the primary sexual cell becomes separated. Its genealogy, the germ-track, marks a main stock within the whole, from which the primary somatic cells with their descendants are derived like so many side branches. Only the cells of the germ-

track retain the original type of karyokinesis and transmit it to the sexual cells of the growing worm; in the primary somatic cells, on the other hand, the nucleus disburdens itself of a certain part of its chromatin and thenceforth follows a new and completely divergent type in all the mitoses of the body" ("nuclear reduction").

The egg of *Ascaris* is normally surrounded by a glassy shell which renders any displacement of its contents impossible, while if the egg is cut in two its contents flow out and perish; the blastomeres cannot therefore be isolated by any direct means. By taking advantage of a number of fortunate circumstances, however, Zur Strassen has succeeded in throwing light on the prospective potency of the blastomeres even in the case of *Ascaris*. The first of these favourable circumstances (cf. 1898) is the occurrence of monster eggs [X, 7], which are already formed in the maternal organism by the coalescence of two or more eggs. Many females have a more or less marked tendency to produce such monster forms even in normal circumstances; but eggs can always be induced to coalesce in any quantity by means of a low temperature. Under the effect of cold the glassy shells become soft and gelatinous; those lying close together begin to communicate by means of passages like canals, and the plasm of the eggs, exhibiting curious streaming processes, also becomes continuous. If the eggs coalesce in the early stages giant eggs arise, elliptical in shape or somewhat like an hour-glass, which behave as regards impregnation like a single egg, that is only admit a single spermatozoon. On the other hand more mature eggs which have already acquired a shell admit two or more spermatozoa, and often exhibit, like those impregnated before coalescence, the well-known polyspermy-polyasters. At this stage they may perish, though many continue to develop and give rise to partial, or complete twin forms; in this case two embryos with like poles grow together, and in all probability the double number of cells is produced symmetrically from an originally simultaneous division into four. The normally impregnated double forms may, however, produce enlarged simple forms which, except in the size of the cells, correspond with normal single embryos in all respects, e.g. in the number of cells, condition of nuclear reduction, form, duration of development and vitality. Development continues until the complete worm is produced.

The second fortunate circumstance of which Zur Strassen made

use is the more or less considerable constriction in space which the monster egg undergoes owing to the elongated hour-glass form of the shell, particularly when the longest axes of the eggs meet almost at a right angle [X, 9]. This enabled Zur Strassen (1903[2], 1906) to show that the blastomeres strive to reach their predestined position in an active manner, and generally succeed in doing so, even when the external conditions of pressure, and particularly their mutual pressure relations are altered. In this case it often happens in the monster forms which are bent at a right angle that certain parts of the egg are unable, during violent movements, to pass through a constriction, and so break off. Such isolated groups of blastomeres develop only into those parts of an embryo to which the blastomeres would have respectively given rise in the intact embryo [X, 10].

(*b*) *Nemertina.*

The nemertines of the species *Cerebratulus* have eggs which can be directly cut in pieces. The normal development of *Cerebratulus lacteus* has been described by E. B. Wilson, who was also the first to make experiments with it. The first furrow divides the egg into two equal parts (right and left side of the body). The further cleavage stages depart from perfect symmetry owing to a displacement of the blastomeres, and, like *Ascaris*, present a "spiral cleavage." A "pilidium larva" arises which develops at the animal pole a long apical organ, and at the opposite pole where the egg was originally attached an archenteron and two ciliated lobes.

If the animal cap which lies peripherally about the nucleus was cut off during the coalescence of the sperm nucleus and egg nucleus, or during the first cleavage complete pilidia with an apical organ were nevertheless obtained (N. Yatsu 1904) [XI, 1]: the plasm which determines this organ must therefore extend still further towards the equator in the form of a ring. This supposition is supported by the double forms of apical organ and enteron [XI, 1*d*] which are not seldom produced by this operation.

If the two halves of the egg are cut apart during the first cleavage, each half is able, like the halves of eggs in earlier stages, to develop a diminutive but otherwise perfect pilidium. The mode of cleavage is however different since it departs more and more from that of a diminutive entire egg, in proportion to the age of the egg at the time of the operation, and resembles with increasing closeness

that of half an egg (Ch. Zeleny 1904[5]: *Cerebratulus marginatus*) [XI, 2—4].

"Isolated blastomeres of the two- or four-cell stage segment, not as wholes but typically, as if the missing blastomeres were present. .....They give rise as a rule to blastulas, more or less widely open at one side, or in extreme cases to nearly flat plates. But all these forms may produce pilidia, of which those from the cup-shaped open blastulas may be normally formed, while those from the plate-forms are usually (always?) asymmetrical" (E. B. Wilson 1903: *C. lacteus*: also E. B. Wilson 1903, and Ch. Zeleny 1904: *C. marginatus*).

"Larvae developed from the upper (i.e. animal pole) quartet of the eight-cell stage always possess an apical organ and lack an enteron, those developed from the lower quartet always possess an enteron and lack an apical organ, while those developed from lateral four-cell groups containing two cells of the upper and two cells of the lower quartet, always possess both apical organ and enteron.

"Larvae developed from the upper four cells of the 16-cell stage lack an enteron but possess an apical organ and blastocoel. Those developed from the lower twelve cells have a large enteron but no apical organ or blastocoel" (Ch. Zeleny: *C. marginatus*).

Fragments of blastulae behave in a similar manner, since they only furnish either certain groups of organs or perfect diminutive forms according to their original position in the egg [XI, 5] (E. B. Wilson: *C. lacteus*; Ch. Zeleny: *C. marginatus*). In all larvae the size of the cell in the ectoderm, the prototrochal region and the mesenchyme remains the same, so that the size of the embryo corresponds to the number of cells and not to the size of the cell (E. B. Wilson 1903[1]).

(*c*) *Annelida.*

Of peculiar interest are the experiments on pressure carried out by E. B. Wilson (1896) on the eggs of *Nereis*, of which he had previously traced the normal "cell-lineage" [X, 16]. "In the normal development of *Nereis* the archenteron arises from four large cells or macromeres (entomeres), which remain after the successive formation of three quartets of micromeres (ectomeres) and the parent-cell of the mesoblast. After the primary differentiation of the germ-layers the four entomeres do not divide again until a very late period (free-swimming trochophore) and their substance always

retains a characteristic appearance differing from that of the other blastomeres in its pale non-granular character and in the presence of large oil-drops. If unsegmented eggs be subjected to pressure, as in Driesch's echinoderm experiments, they segment in a flat plate, all of the cleavages being vertical. In this way are formed eight-celled plates in which all of the cells contain oil-drops [X, 7]. If they are now released from the pressure, each of the cells divides in a plane approximately horizontal, a smaller granular micromere being formed above, leaving below a larger clear macromere in which the oil-drops remain. The 16-cell stage therefore consists of eight deutoplasm-laden macromeres and eight protoplasmic micromeres (instead of four macromeres and twelve micromeres, as in the usual development). These embryos developed into free-swimming trochophores containing eight instead of four macromeres, which have the typical clear protoplasm containing oil-drops" (E. B. Wilson 1896).

The particular interest of this experiment lies in the fact that the nuclei of four of these eight endoderm cells were originally or normally destined to form the first quartet of micromeres from which ectodermal organs, namely the apical ganglia and the prototroch, should have developed; thus the future destiny of the nuclei cannot have been impressed upon the cells, but only the distribution of the cytoplasmic substances.

Wilson has more recently found (1904), by means of direct isolation with the knife, that in the annelid *Lanice* the first two blastomeres, on separation from the rest, develop in accordance with their prospective significance in normal development, i.e. simply as the corresponding part of the egg. In other words their prospective potency is not greater than their prospective significance. The "posterior" cell develops a segmented larva with prototroch, an asymmetrical pretrochal region and an almost typical metameric bristle-forming region, while the "anterior" cell produces apical organ, prototroch and pretrochal region, but no metameres.

Lillie (1902, 1906) found differentiation without cleavage in his parthenogenetic eggs of *Chaetopterus*. Nuclear divisions occur which, however, are not followed by divisions of the cytoplasm, so that finally all the chromosomes again lie together: their number corresponds to the sum of all the chromosomes which would occur in the cells in the case of normal fertilisation. Nevertheless the embryos continue to develop until they produce forms resembling trochophores.

(*d*) Kingcrab (*Limulus*).

The only experiments among the arthropods appear to be two series made by Patten (1894, 1897) on *Limulus*. Eggs of the kingcrab (*Limulus polyphemus*), obtained by fishing them in the natural state, show cleavage on every side. But if eggs are placed in a glass vessel they adhere firmly to the bottom, and, after artificial impregnation, only the upper part segments. If the eggs are then turned so that the part previously below is now above, as in the experiments made by Patten (1894), this part also divides into blastomeres; thus the formation of cleavage lines appears to be checked by the "yolk" accumulated below.

In his second communication Patten (1897) describes the further development of eggs with injured blastomeres, which afterwards became complete by "postgeneration." Possibly it is merely a case of slower development of the injured blastomeres, as in similar cases in the frog's egg, originally described by Roux as instances of postgeneration.

5. *Mollusca.*

Among the molluscs experiments with regard to the development of isolated blastomeres have been made with the scaphopod *Dentalium* (E. B. Wilson 1904), and several gasteropods, namely *Ilyanassa*, *Urosalpinx*, *Anachis* (2 sp., Crampton 1896), *Aplysia* (Fŭjita 1896), and *Patella* (E. B. Wilson 1904[3]).

All the eggs of molluscs which have been studied are distinguished by an early differentiation of the several cleavage cells. They exhibit a "spiral" type of cleavage and, with the exception of *Patella*, form on the appearance of the first furrow a so-called "yolk-lobe." This is attached to one of the blastomeres, and during each pause between the divisions unites with the corresponding cell to form a sphere, so that this cell appears larger than its fellow by the volume of the lobe.

The smaller blastomeres situated at the original nuclear pole form the ectoderm (ectomeres), the larger blastomeres, opposite these, form the endoderm (entomeres), and the cell to which the yolk-lobe is allotted furnishes the intermediate organs ("somatoblasts"). Gastrulation takes place without typical invagination; an anus is formed by perforation at the entomere pole, and is followed by the

appearance of an oral aperture on the equator, which is surrounded by three ectodermal ciliated rings.

The "pro-trochal" and "post-trochal" regions become narrowed to a point, a long apical, and a shorter anal tuft of cilia is developed, and thus the "trochophore larva" arises.

"The *Dentalium* egg [XII, 1—10] shows from the beginning three horizontal zones [XII, 1], an equatorial pigment-zone and two white polar areas. During cleavage the pigmented zone is allotted mainly to the entomeres, the upper white area to the ectomeres, the lower white area to the first and probably also to the second somatoblast.

"At the first, second and third cleavages the lower white area temporarily passes into the 'yolk-lobe' or polar lobe [XII, 2, 3].

"Removal of the first polar lobe leads to a symmetrical cleavage without the subsequent formation of polar lobes, and to the formation of a larva devoid of post-trochal region and apical organ. Removal of a portion of the first lobe produces a larva with reduced post-trochal region, and with or without an apical organ. Removal of the second polar lobe produces a larva without a post-trochal region but with an apical organ.

"The lobeless larvae undergo no metamorphosis, form no foot, shell gland or shell, no mantle folds, no pedal ganglia, apparently no mouth, and probably no bands of coelomic mesoblast."

The isolated *AB* half, or either the *A*, *B*, or *C* quarter [XII, 4] produces a closed larva, closely similar except in size to the lobeless ones. The isolated *CD* half or *D* quarter produces a larva possessing a post-trochal region as large as in a normal larva and an apical organ; it dies without undergoing metamorphosis. The *CD* half, from which the second polar lobe is removed, produces a larva like that from an *AB* half, but possessing an apical organ. The isolated micromere *Id*[1] produces a mass of ectoblast cells bearing an apical organ, while *Ia*, *Ib*, *Ic* produce no apical organ.

"Enucleated fragments of fertilised eggs, containing the lower white area, pass alternately through periods of activity and quiescence corresponding with the division-rhythm of the nucleated half, and produce the polar lobes as if still forming part of a complete embryo. The same is true of the isolated polar lobes.

"The foregoing observations demonstrate the pre-localisation of specific cytoplasm stuffs in the unsegmented egg, and their isolation

[1] *Ia*, *Ib*, *Ic*, *Id* are the micromeres of the eight-cell stage constricted off from *A*, *B*, *C*, *D* respectively towards the animal pole.

in the early blastomeres. The lower white area contains such stuffs as are essential to the formation of the apical organ and the complex of structures forming the post-trochal region, including the shell gland and shell, the foot, the mantle folds, and probably the coelomic mesoblast. These stuffs are contained in the first polar lobe, but the second polar lobe no longer contains what is necessary for the formation of the apical organ. Progressive changes therefore occur in the original distribution of the specific cytoplasmic stuffs."

Crampton's account of the snails he investigated (in particular *Ilyanassa obsoleta*) runs as follows:

"*A.* The cleavage of isolated blastomeres of the normal two-cell stage is in all essential respects the same as in a corresponding half of the complete embryo, but the micromeres of the fourth generation continue to lie at the surface, and do not pass into the interior as in normal development. In the larger half embryo, however, a cell corresponding in origin and appearance to the mesoblast pole-cell (*d*4) arises from the large macromere *D*. Development continues for but a short time, after which the cells become rounded and disintegrate.

"*B.* Blastomeres of the four-cell stage segment as if still forming a quadrant of the normal embryo. Three micromeres are successively formed, and their later divisions are as in the normal embryo. Large quarter blastomeres (*D*) do not segment long enough to produce a fourth micromere (corresponding to the mesoblast pole cell).

"A fourth cell arises from the other macromeres (*A*, *B*, or *C*), and continues to lie at the surface for a time. The ectomeres finally overgrow the entomeres, and a partial circle of cilia is developed, but the embryos never live more than four days. $\frac{3}{4}$ embryos develop precisely like the $\frac{1}{2}$[1].

"A $\frac{3}{4}$ embryo (three $\frac{1}{4}$ blastomeres) develops as if the missing quadrant were present.

"*C.* Micromeres of the first quartet ($\frac{1}{8}$ blastomeres) segment as if in place in the complete embryo. A micromere remaining in connexion with a macromere ($\frac{7}{8}$) forms a $\frac{1}{4}$ embryo. Two micromeres duplicate the single micromere condition. Macromeres of the eight-cell stage, however, do not segment on isolation, thus marking the limit of further independent segmentation of the yolk-bearing cells.

[1] The denominator indicates the number of blastomeres in the stage operated upon, the numerator the number of the remaining blastomeres, the development of which is traced further.

"*D.* Isolated cells of later stages do not divide. However if several cells remain united, segmentation may proceed for a time. Thus a $\frac{5}{16}$ embryo will simulate a $\frac{1}{4}$ ($\frac{4}{16}$) embryo; $\frac{7}{16}$ or $\frac{9}{16}$ will develop nearly like the $\frac{1}{2}$ ($\frac{8}{16}$). The fate of all these fragments is the same, viz. disintegration.

"*E.* If the yolk-lobe be removed from the 'trefoil' stage the two equal cells remaining develop in a manner similar to the normal development of *Physa*, although not reversed. The second cleavage divides each of the original cells, giving a four-cell stage of four equal blastomeres. Four successive quartets of 'micromeres' are produced, as in the normal egg, but the cell of the fourth group, derived from the macromere *D*, has the same size and appearance as those derived from *A*, *B*, and *C*. There is none that can be identified with a mesoblast pole cell. Embryos of this kind live several days, develop a band of cilia, and swim quietly around, but never reach the veliger stage. The isolated yolk-lobe itself never divides."

The normal development of *Patella* [XIII] is distinguished from that of *Dentalium* and *Ilyanassa* by the absence of the yolk-lobe. It is characterised by a "ctenophore" stage with 16 ectodermal primary "trochoblasts." In the absence of localisation of material in the lower region, none of the first blastomeres appear predestined for the formation of the apical organ. Each isolated blastomere of the two-cell stage forms an apical organ and a prototroch; the cleavage [XIII, 7*b* and β] is either open or closed and the form of the larva varies accordingly. Isolated $\frac{1}{4}$ blastomeres give rise to an embryo which in the gastrula stage produces four instead of sixteen primary trochoblasts, at least two secondary trochoblasts and an apical organ. Herbst's method of isolating blastomeres by placing them temporarily in water devoid of calcium is so easy to apply that even the blastomeres of a later stage may be isolated by its means. One eighth micromeres produce pearshaped larvae, bearing at one end an apical organ and at the other a group of four primary and two secondary "trochoblasts"; these do not gastrulate. On the other hand, $\frac{1}{8}$ macromeres produce closed embryos which gastrulate and bear at one end one or two secondary "trochoblasts," and at some other point a small group of feebly ciliated cells, which probably represent the pre-anal ciliated cells of the normal larva. Isolated $\frac{1}{16}$ macromeres behave in the same manner, but form no trochoblasts. Isolated primary trochoblasts distinguished as 1^2, divide twice and produce four typical ciliated prototrochal cells; isolated first products of the

primary trochoblasts $1^{2.1}$, $1^{2.2}$ divide once and form a pair of typical prototrochal cells $1^{2.1.1}$, $1^{2.1.2}$, and so on. If these are isolated they form typical prototrochal cells, but undergo no further division. Isolated micromeres 1^1 which are not primary trochoblasts produce embryos with an apical organ and opposite to this two secondary trochoblasts. Isolated division products of these cells differentiate either into typical sensory cells of the apical organ, into secondary trochoblasts, or into but slightly differentiated ectoblast cells.

No experiments have been recorded on the lamellibranchs, though from the resemblance of the egg of *Unio* to that of the gasteropods similar behaviour may be expected.

The cephalopods also have not been experimentally studied. Their eggs, which are rich in yolk, enable us to distinguish different sides, as we have already mentioned with regard to the promorphology of animal eggs. If the first anal development is traced further it appears that the different sides of the later embryo may be referred to the differences already recognised in the unsegmented egg; the narrow pole of the elongated egg corresponds to the future dorsal side, the broad pole to the ventral side, the flat part of the egg to the posterior region, and the arched part to the anterior region of the embryo.

The furrows are all arranged symmetrically about a plane which is given by a line uniting the two poles, and another line vertical to it uniting the right and left flanks, which eventually becomes the future median plane of the embryo. At the same time the first furrow is said always to separate the right side of the body from the left, and the second furrow the anterior part of the body from the posterior (Watasé 1891).

6. *Prochordata and Pisces.*

The tunicates present normally a bilaterally symmetrical type of cleavage [XIV, 1—7], which in the case of *Clavellina* (according to Castle 1896) already enables us to distinguish the future sides of the larva. The first furrow separates the right side of the body from the left, the second divides, somewhat unequally, two larger "anterior" cells from two smaller "posterior" cells; later furrows separate larger "entomeres" from smaller "ectomeres"; the gastrulation is a process of typical invagination. The

ectodermal cells at the blastoporal lip become nerve and sensory cells (eye, otolith), while those at the anterior end form three adhesive papillae. The endodermal cells below the anterior lip become constricted off to form the chorda which afterwards runs along the dorsal side below the nerve cord; at the posterior lip mesoderm is constricted off. The remaining endodermal sac acquires a dorsal oral aperture situated anteriorly to the sensory organs. The posterior part of the animal grows out in a long tail with a ventral depression which contains no further endodermal organs. In these Ascidian larvae which escape from the egg envelope the structure of the chorda, previously many layered, is now that of a single series of cells.

Chabry (1887) first experimented on *Ascidiella aspersa* by pricking with fine needles and with various complicated instruments. If a blastomere of the two-cell stage was killed by pricking the remaining blastomere nevertheless produced a small embryo which presented on the whole the form of an entire embryo, but might lack certain organs such as the eye, otolith, or adhesive papillae [XIV, 8]. Driesch opposed the interpretation of these forms as "half forms" since it is not the right or left, anterior or posterior half of the embryo which is lacking but simply certain organs of secondary importance. Driesch (1895[1]) repeated the experiments on *Phallusia mammillata*, and found that there was no relation between an injury to a particular blastomere and the defects produced [XIV, 9]. At the same time the cleavage was that of a diminutive perfect form, not that of an open fragment. In any case the morula and the gastrula from the eight-cell group, form a complete and not a semi-gastrula. The embryo possesses, as in normal cases, a chorda which at first consists of several layers and afterwards of one layer. "An eye spot is almost always present, an otolith very seldom, though both are rudimentary in comparison with the corresponding formations of normal larvae. Adhesive papillae have also been very seldom observed, even then, only one generally appears, seldom the normal number of three." Driesch refers the defects to general injury from the operation, which consisted of cutting or shaking; he obtained the same defects by exposing normal intact eggs for some time to direct sunlight, or placing a large number of them in a small glass in impure water. Crampton (1897) after making experiments with *Molgula manhattensis* agrees with Driesch's interpretation.

On the other hand Conklin (1905[1–8], 1906) not only observed a typical "mosaic" development in *Cynthia* (*Styela*) *partita* and

Molgula manhattensis, but in the case of the half or quarter larvae obtained respectively from half or quarter embryos he saw no later regulation tending to produce complete forms. Thus Chabry seems to have been right.

The normal development of ***Amphioxus*** [XIV, 10—13] is known to resemble closely that of the tunicates. While the first two furrows pass through the nuclear pole, the third is equatorial and produces a somewhat unequal division, four animal "micromeres" being formed. A typical morula, blastula, and gastrula stage leads to the development of a bilaterally symmetrical embryo. The nerve cord is formed dorsally from the ectoderm, the chorda from the endoderm; the mesodermal structures on each side are likewise formed from the endoderm; these, growing longitudinally, and becoming transversely constricted off, furnish the later "somites." The mouth is perforated ventrally (and on the left side) at a very late stage; the anus occupies the position of the ancient "blastopore." By means of shaking the blastomeres can be caused to fall apart within the egg membrane. Isolated blastomeres of the two- or four-cell stage (Wilson 1893) [XIV, 11*a*, 12*a*], as well as separate blastomeres of the eight-cell stage (Driesch), give rise to diminutive perfect embryos. The cleavage is either closed from the beginning or the cleavage cells form a flat disc, which however is also produced by normal embryos. Intact eggs, of which the blastomeres were brought to lie in one plane by means of gentle shaking gave analogous results (Morgan 1896[1]). Larvae of ***Amphioxus*** developed from half eggs contain, according to Morgan, about two-thirds the number of cells of a normal larva, the larva in building up its organs showing a tendency to form the normal number of cells. The archenteron of the half egg larva contains in cross section about two-thirds the normal number of cells, and the ectoderm also two-thirds; the chorda and the spinal cord contain the full normal number; quarter larvae behave in exactly the same manner with regard to these several organic systems, but their entire number of cells is only half the normal number.

If two or more blastomeres are incompletely separated by the shaking, double embryos like Siamese twins may arise, though their axes show no constant relation to each other [XIV, 13].

Bataillon (1901[1]) found that the eggs of ***Petromyzon planeri***, obtained towards the end of the breeding season from females which had previously produced normal embryos, frequently gave two embryos each. A seemingly voluntary "blastotomy" took place,

resulting in the second blastomere becoming completely constricted off. It was conjectured that this was caused by osmotic forces, owing to the sudden transference of the eggs into the water from the sexual duct, of which the contents are greatly thickened towards the end of the breeding season, and this was confirmed experimentally by Bataillon. The experimental blastotomy was achieved by using solutions of salts or of sugar which were isotonic to a one per cent. solution of common salt. The eggs remained for a certain period in the fluid selected and were then replaced in the normal medium. In this case well marked multiple and complete double forms were observed.

In the case of the cyclostomes, which resemble the tunicates and *Amphioxus* in their mode of cleavage and development, diminutive intact embryos may arise from each of the two first blastomeres and also from individual blastomeres of the later stages [XIV, 14—16].

By means of blastotomy in the roach (*Leuciscus rutilus*) Bataillon also obtained monstrous double forms with discoidal, that is partial, cleavage [XIV, 32—34].

Morgan (1893) removed from the egg of the bony fish *Fundulus* one blastomere of the frequently unequal two-cell stage by pricking it with a needle and then causing it by pressure to flow away; the remaining blastomere at first divided as if the other were still present but more slowly. This is also the case with the two succeeding furrows, but then the homology disappears and the single blastomere finally gives rise to a complete embryo which is smaller than the normal. It is shorter by a quarter of its length, and also differs in size according as it arose from the larger or smaller blastomere. The yolk which would have supplied the blastomere removed is naturally at the disposal of the remaining blastomere so that the size does not correspond to that of half an egg [XIV, 30]. No difference can be observed between operated and normal eggs as regards the size of the nuclei or (in cross sections) of any of the organs. The smaller number of cells in the entire embryo, which may be somewhat smaller than the normal, finds expression in the abbreviation of its length.

A number of experiments have been made with regard to the so-called "theory of concrescence" (His). The cleavage of the teleosteans and some other fishes is partial and discoidal [XIV, 22—26].

The "germinal disc" or "blastoderm" has the form of a round and

somewhat arched dome and possesses (in the case of the salmon) a thickness of 8—10 cells. Below the blastoderm lies the flat cleavage cavity and the blastoderm rests on the periblast only at its margin. The periblast is represented by a zone passing round the blastoderm and containing protoplasm and it is continued by a narrow band along the base of the cleavage cavity. Below, the periblast passes without sharp delimitation into the yolk sphere.

Gastrulation begins at one spot in the margin of the blastoderm. Here an invagination commences to form, a kind of inflection so to speak in the margin of the blastoderm [XIV, 23]. Thus a second layer arises which is termed the "lower layer or primary endoderm." "During the formation of the lower layer the whole blastoderm becomes thinner and at the same time extends further in all directions over the yolk sphere" [XIV, 24].

Meanwhile, however, the border of the blastoderm only becomes thinner to a certain extent; the blastoderm thus seems to be relatively thickened along the whole border, and the thickened border is termed the germ wall [XIV, 25]. This passes at the posterior margin of the blastoderm into the embryonic rudiment. "When the lower layer has been formed and the germinal wall raised up, the germ layers can be distinguished. The lower layer or the primary endoderm becomes differentiated into the mesoderm, chorda and endoderm (secondary endoderm, intestinal epithelium)."

The remainder of the blastoderm may henceforth be termed the ectoderm. The further development consists of the extension of the embryonic structure, and the envelopment of the yolk sphere by the blastoderm which pushes before it the germinal wall, steadily decreasing in thickness. Finally the borders of the germinal wall meet in the (future) median line of the embryo, and the blastopore disappears completely [XIV, 26]. His believed that by this union the right and left parts of the embryo, which were formed separately now meet secondarily for the first time.

This "theory of concrescence" is refuted by experiments in which one or even both sides of the germinal wall were separated from the embryo, and yet the growth of the embryo proceeded almost unchecked, since the trifling defects of mass in the separated side might easily be referred to the diminished supply of material. Experiments of this nature have been carried out with equal success by Katschenko (1888) on a selachian (*Pristiurus*), by Morgan (1895[7]) on the bony fish *Ctenolabrus*, and by Kopsch (1896) [XIV, 31] on *Salmonidae*;

Rückert, who has also studied *Pristiurus* and was able in his experiments to rear the embryos to a later stage, is inclined to suppose from the defects which these embryos exhibited that the germinal wall does to a certain extent share in the formation of the hindermost part of the body by concrescence. Kopsch (1898) showed in the case of *Scyllium* that the symmetrical development of the posterior end was only prevented when the germinal wall was cut off very close to the tail lobe [XIV, 17, 18]. Francis B. Sumner has recently made extensive experiments on the position of the embryo with regard to the original egg axes [XIV, 27—29].

That the "yolk" in fishes does not play the specific part of the so-called yolk-lobe in molluscs was shown by Morgan (1893) in the eggs of *Fundulus* from which more than a half of the yolk was removed without causing any disturbance of the embryonic development. Cleavage under pressure also exerted no influence on the development.

The experiments were made by means of electro-cautery or with glass needles; the latter if broken off and left sticking in the wound made a lasting mark.

The species employed were *Exocoetus sp.*, *Salvelinus fontinalis*, *Batrachus tau*, *Fundulus heteroclitus* and *F. majalis*. Of these the last gave the best results, for *Exocoetus*, though favourable on account of its rapid development, could seldom be obtained, and the great eggs of *Salvelinus* and *Batrachus* were unfavourable on account of their slow development. It appeared that the head of the embryo came to lie at the animal pole, but that displacements might occur as the results of obstacles and that the severance of the germinal ring even on both sides did not prevent the development of a complete embryo.

The eggs of *Amia calva* differ from those of most other true fishes in their holoblastic cleavage. These elongated eggs adhere to water plants in any position as regards the force of gravity [XIV, 1], the vegetative darker pole serving as the point of attachment. The division of the egg into this darker pole and the lighter animal pole is quite independent of weight even before impregnation (H. Dean King), so that the eggs remain in any position without becoming rearranged. If the eggs are placed, during the appearance of the first or second furrow, so that the cleavage plane is vertical, it may be observed that the future median plane of the embryo stands in no definite relation to the first furrow, though the head always comes to

lie at the animal pole of the egg (Whitman and Eyclesheimer) [XIV, 20—21].

Jan Tur (1906) experimented with radium rays on the eggs of *Scyllium canicula* and found that by this means the development of the nervous system, and to a less extent that of the rest of the ectoderm, could be checked without affecting the further differentiation of the peripheral blastoderm.

In the later embryonic stages of selachians Braus (1906[1]) deals with the question as to whether the formation of the skeleton is dependent on the muscular system. In *Scyllium canicula* and *Pristiurus melanostomus* it appeared that the artificial elimination of the muscular system was no hindrance to the development of the cartilaginous pieces. On the other hand the skeletal rods which belong to the secondary basalia of the fins could not of themselves differentiate in the general blastema in which the radii of the fins of *Scyllidae* appear as concentration centres of the skeletogenous material. This differentiation appears to be produced by an impulse from the metapterygium (primary basals) and more exactly from the radii first formed (that is the most preaxial radii).

7. *Fourfooted vertebrates* (*tetrapods*).

(*a*) Tailed batrachians (*Amphibia urodela*).

In their mode of cleavage the eggs of *Amphibia* stand midway between those of *Amphioxus* and the bony fishes. The cleavage is complete but unsymmetrical. Among the tailed batrachians (in particular *Triton cristatus* and *T. taeniatus* [XV, 1]) experiments have been made on the fate of the blastomeres by a method first suggested by O. Hertwig which consists in constricting them off with a fine hair. Complete division of the egg without injury was first achieved by Endres (1895) who first constricted the egg to a moderate extent and then severed the remaining neck of material with a hot needle; Herlitzka (1895, 1897) employed a particular apparatus which produced very gradual constriction. Both investigators, by constricting the egg along the first furrow, obtained sometimes two complete embryos developing uniformly, and sometimes forms differentiating at different rates and to different extents. No attention however was paid to the latter until Spemann showed by fresh experiments that two typically different cases were here presented. Spemann (1901)

laid a thread in the first furrow at the time of its appearance without constricting the egg; the embryo developed normally and the thread remained simply as a mark to indicate the position of the first furrow. It then appeared that the embryo did not always assume the same position with regard to the plane of the thread, i.e. the first furrow does not always divide the egg in the same manner.

In this case there are two principal types of cleavage; in the majority of cases, namely in from two-thirds to three-quarters of all cases, the first cleavage plane becomes a frontal plane [XV, 2*a*], in a considerable minority of cases, namely from one-quarter to one-third of all cases, it becomes a median plane [XV, 2*a*, *b*].

If eggs of the latter type are completely divided by constriction in their later stages, the morula or blastula or even the two-cell stage, two diminutive complete embryos arise [XV, 2*c*]. In those eggs on the other hand where the first furrow corresponds to a later frontal plane, this furrow separates a dorsal from a ventral blastomere [XV, 2β]; if the thread is drawn tight during later stages [XV, 2γ] only the dorsal half develops to a complete embryo, while the ventral half produces no further differentiation in the three primary germ layers, neither a medullary plate, nor a chorda, nor any composite organ; but when gastrulation is completed it remains almost entirely stationary [XV, 2δ]. Similar results were obtained by Spemann on constricting in the gastrula stage [XV, 3*a*]: in the case of frontal constriction that part of the body derived from the originally ventral blastomere differentiated various regions such as tail, auditory vesicles, primary vertebrae, pronephric duct, but remained without chorda and eye vesicle; the dorsal half on the other hand forms a complete embryo [XV, 3β]. By constriction without complete severance Spemann obtained double forms of the anterior end in the two-cell and blastula stage and also in the first gastrulation stages [XV, 4, 5]; in the succeeding "neurula" stage this power appears to be extinct(?) and the embryos perish.

Spemann (1906) removed almost the whole animal half of the germ from *Triton taeniatus* at the very beginning of gastrulation by cutting it out with glass needles, and then caused the egg to heal up again while turned through an angle of 90° or 180°; in several indisputable cases these eggs produced normal embryos.

When embryos were constricted transversely shortly before or after the appearance of the medullary plate the anterior and posterior half each developed as though it were still connected with the other

half. In the former case it appeared that the rudiment of the medullary folds is situated in the anterior end before these actually become visible. The anterior part differentiated eye vesicles; the posterior part formed a medullary groove, but no folds. All these transverse fragments perished without further development. With regard to the size of two complete embryos each developed from one blastomere of the two-cell stage, Herlitzka (1897[1]) states that they are larger than half an embryo from an intact egg. The size and geometrical form of the elements (cells and cell-nuclei) in each stage of differentiation are the same as in normal embryos of corresponding stages. For in these also the size relations of the elements with a definite histological differentiation remained unchanged, while with every histological change the size and outer form of the elements also change (1897[2]). The diameters of the chorda and medulla are the same in embryos from half eggs and intact eggs; consequently the smaller number of cells in the former can only find expression in the diminished length of the embryo. On the other hand the diameter, and therefore the number of cells, is smaller in transverse sections of the gut and myotomes in the case of half embryos.

Oskar Levy (1906) also observed self-differentiation of certain organs in the later stages of embryonic development in newts. If for example a part of the fore brain with half the eye rudiments was removed, two eyes were developed in immediate contact with the original wound. Scattered brain and eye rudiments continued to develop, but when no eye cup was formed the pigment layer was also absent. Isolated sections of the heart separated by constriction were able to develop in complete independence of one another. The auditory organ also appeared to possess a certain power of self-differentiation. Rudiments of the olfactory fossae when removed did not form again, the brain after asymmetric operations showed a certain power of regulation, but might be mechanically prevented from assuming a symmetrical form by the invagination of the eye vesicle into the wound. Pigmentation occurred independently of the development of the lateral line, the vagus nerve, the heart and the circulation.

The formation of the several parts of the eye has been made the subject of a thorough investigation, and here we meet with cases of independent differentiation. Spemann (1904, 1905) has studied the formation of the lens.

"The experiments consisted in removing from the embryos of

Triton taeniatus the head of the primary eye vesicle together with the primary cells destined for lens formation, or in somewhat later stages the outer part of the eye cup together with the first rudiment of a lens which had just become visible [XV, 6]. The result of these experiments was as follows:

"1. In many cases the more or less regenerated eye rudiment remained below the surface, without touching the epidermis; the rudiment did not produce a new lens rudiment [XV, 6*a*].

"2. In a number of cases the regenerated eye cup reached the epidermis where a new lens arose which developed further in a normal manner [XV, 6*b*].

"In one case where the eye cup could not reach the epidermis a small distinct lens bud developed on the upper margin of the iris such as Colucci observed after mutilation of the eye and G. Wolff after complete extraction of the lens."

W. H. Lewis (1905) experimented on the axolotl (larva of *Amblystoma*), devoting his attention chiefly to the origin of the cornea. The operations were performed with fine scissors and chloroformaceton as an anaesthetic. A normal cornea did not develop without the eye; its size varied with the size of the eye and the extent of the surface of contact of the eye with the skin. If the eye happens to be separated from the skin by mesenchyme no cornea develops. The optic vesicle is capable of producing a corneal formation without a lens. This is also true of the lens without the optic vesicle provided that it reaches the skin in the same manner as when the vesicle is present. The cornea may also be formed from foreign ectoderm. If the remainder of the eye is removed after the formation of the cornea the latter degenerates and disappears entirely. There is thus no doubt that independent differentiation occurs in the case of the cornea and lens, a fact confirmed in other groups of animals (see below, *Anura*).

The metamorphosis of the axolotl larva to the *Amblystoma* occurs, according to Loeb (1896), even if the spinal cord is completely severed; thus developmental processes must take place in very late stages independently of functional activity.

(*b*) Tailless batrachians (*Amphibia anura*).

The prospective significance and potency of the blastomeres of the frog's egg [XV, 7]—which through Roux' experiments led to the experimental treatment of this whole question—has been studied in a

number of experiments. The results appear at first directly to contradict one another, but on the analogy of the differences discussed in the case of *Triton*, and by combining several modes of experiment (Morgan) they may be made to bear a consistent interpretation.

Roux' experiments (1895[1]) consisted in pricking one blastomere of the two-cell stage [XV, 8*a*] with a hot needle; from the uninjured blastomere (after "half" cleavage, blastulation and gastrulation [XV, 8*b*]) he obtained half forms which generally corresponded to one side of the body, "*hemiembryones laterales*" [XV, 8*c*], or more seldom such as did not always correspond to one side, and these he termed "*hemiembryones anteriores*." From this it must be concluded that the first furrow normally corresponds sometimes to the median plane, sometimes however to a transverse plane, as in the case of *Triton*, but with the difference that in the frog the first furrow generally represents the median plane. In these cases the second furrow lies transversely [XV, 8*d*], as in the minority of cases the first furrow, and by operating in the four-cell stage [XV, 9*a*] the form termed by Roux "*hemiembryones anteriores*" [XV, 9*b*] as well as quarter defects (Vierteldefekte) are obtained by pricking one blastomere only. Corresponding "*hemiembryones posteriores*" do not appear to have been obtained with certainty while the "laterales" and "anteriores" of Endres (1894), Walter (1895), K. Ziegler (1902) and Morgan (1894[2]) could be tested by Roux' methods.

It is possible that the "*hemiembryones anteriores*" must be described as "dorsales" as in the case of *Triton* and that the absence of typical "ventrales" must be referred to the fact that these show no further differentiation. In contrast to Roux, O. Hertwig obtained half sized perfect forms from one blastomere, but Roux regards them as the result of a sort of posthumous regeneration of the absent half or "postgeneration"; this also occurred at a later period in his *hemiembryones* but ran a very rapid course. According to the descriptions of these processes by Roux, Endres, Walters, and particularly Kŭrt Ziegler (1902), it is often, but not always, a case of slower after development of the injured blastomere when its nucleus had not been killed but only injured by pricking. A reversal of the experiments shows that the half forms are determined by the inability of the uninjured blastomere to so rearrange its materials as to produce a form proportional to the whole if its position remains unchanged and if it is hampered on one side by the adjacent injured matter. In continuation of the experiments of Pflüger, mentioned

above, to discover whether the development of the egg is dependent on the force of gravity, O. Schultze (1894) attempted to determine the influence of a reversed position of the eggs in the two-cell stage, and prevented them from a general rotation by compression. Eggs thus fixed between two sheets of glass with the white pole turned upwards instead of the normal black one, were left in this position until the beginning of gastrulation and then freed from their constrained position. They produced double forms [XV, 10], from which it must be concluded that each blastomere was totipotent. The manner in which the egg substances become rearranged is made clear by the sections which Wetzel (1895) prepared from double forms artificially produced in this way. A descent of the heavy yolk [XV, 11] takes place from the vegetative pole, which is turned upwards, along the furrow, while there is a corresponding ascent of the pigmented part along the outer surfaces of the blastomeres. Morgan (1895[6], 1904) combined the process of pricking the blastomere with Schultze's methods of reversal and found that when the white pole of the pricked egg was turned upwards the one uninjured blastomere produced a proportionately diminished perfect form [XV, 12]. In control experiments in which the black pole remained turned upwards typical hemiembryos were produced [XV, 13]. In eggs under the influence of centrifugal force some blastomeres, according to Morgan (1902), often died in later stages (e.g. in the 128-cell stage) and in that case self-differentiation and also perfect forms might result. Much dispute also arose in the case of Amphibia Anura with regard to the "concrescence theory," which has been discussed with reference to fishes. If eggs of these species are injured by pricking, or in some other way, extraovates containing yolk are often formed which prevent the lips of the blastopore from closing. On each of these the end of a rudiment of a vertebral column is formed which was termed by O. Hertwig "*spina bifida*," and previously by Roux "Asyntaxia medullaris." It is doubtful whether this open blastopore can be regarded as a proof of the normal approach of the right and left sides of the germinal wall to form the posterior end, since the occurrence of the two spinal columns accords better with the totipotency of each side.

Small extraovates which had been pierced were used by H. D. King (1902) to determine the place of origin of the embryo in the egg of the toad, *Bufo lentiginosus*; she found that the material lying just above the dorsal blastopore lip forms the middle part of the embryo. Its

anterior end does not reach the centre of the black hemisphere but is formed somewhere between the place where the dorsal blastopore lip first appears and the centre of the black half of the egg.

The embryo thus arises partly in the region of the dark half of the egg, partly on the surface of the light half. A. H. Todd (1904) states of the frog (*Rana palustris*) as the result of experiments made on pricking the dorsal lip of the blastopore: "The embryo develops chiefly over the lower hemisphere; the head is formed in and close beside the region where the dorsal lip first appeared and the tail appears at a point nearly opposite in the lower hemisphere."

King (1905), Spemann, Steinitz, and Streeter have made experiments on the later stages of the frog.

"In order to determine the moment at which the rudimentary material becomes destined to form the optic vesicle, and eventually its several parts, the retina and *tapetum nigrum*, a square piece was cut out of the medullary plate (of *Rana esculenta*), while the walls were wide open, and was then allowed to heal in again in a reversed position:.....embryos were produced with four eyes, two in front in their normal position, two more or less towards the rear, before or behind the auditory vesicles. The eyes varied very much in size....." (Spemann, 1906).

H. D. King (1905) made experiments on the embryos of *Rana palustris*, which consisted in destroying the eye with a hot needle immediately after its development, or the corresponding rudimentary region immediately before its development; she found that the fore-brain was not capable of producing the eye rudiment afresh. In one case a development of the eye occurred unconnected with the nerves and surrounded by mesoderm; a lens was absent; some part of the fore-brain had probably been scattered by the operation. But an optic cup may be formed independently of the connexion with the brain. In opposition to the facts mentioned in connexion with the experiments on *Triton*, a lens is said by King to arise even without contact between the optic cup and the ectoderm; perhaps this is a case of the formation of a lens from the upper margin of the optic cup. E. Steinitz (1906), who made similar experiments on *Rana fusca*, found that no fresh eye rudiments appeared if the experiment was made at the time of the separation of the retina and the outgrowth of the first nerve fibres from the retina. The parts which afterwards stand in a functional relation to the eye, socket (orbital) muscles, foramen, nerve and so on were affected in their development only after the time at which function was established.

The behaviour of the ear rudiment is analogous to that of the eye rudiment. G. L. Streeter (1906) completely removed the ear rudiment [XIV, 14] from young larvae of *Rana silvatica* and found that it did not reappear; amongst the adjacent organs only the ear protuberance was absent [XV, 14*a*]. If ear rudiments were transplanted to other parts of the body they continued to differentiate and even developed ganglia and a connexion with the brain, though no functional activity was observed.

The ear rudiment behaved in precisely the same manner as the limb buds in the earlier experiments of Roux (1904). In these experiments performed on *Bombinator* larvae rudiments of the extremities differentiated in accordance with their original significance without regard to the place of implantation; for example, a rudiment of a fore leg transplanted immediately in front of or upon the hind leg, became a fore leg, and this was also the case when it was inserted in front of the eye.

These transplanted "parasitic" extremities differentiated nerves which established connexion with the central nervous system of the "host." That they are not simply furnished with nerves by this system was proved by Braus, who among other methods employed "aneurogenous" rudiments for transplantation. These are rudiments obtained from larvae from which the back part together with the whole of the rudiment of the spinal cord had been removed, according to a method devised by Harrison [XV, 16]. In these larvae the nerves of the extremities did not develop. In contrast to the transplanted "euneurogenous" rudiments, that is rudiments from embryos provided with nerves, no nerves were differentiated in the "aneurogenous" rudiments.

The capacity for independent differentiation and for the union of tissues of the same kind had already been observed by Born (1894, 1897) to exist in a high degree in frogs' larvae cut in two and caused to heal up in various manners.

Born's method of transplantation was employed by Harrison (1903) in order to investigate the origin of the sensory organs of the lateral line of *Amphibia*. "The anterior (oval) part of a *silvatica* embryo is united with the posterior (caudal) part of a *palustris* embryo, so that a composite individual of normal form is produced."

The lateral line then grows out from the head region of the darker *R. silvatica* component, and this takes place independently of the mutilation or complete removal of the brain or nerves. Bends in the tail directed upwards or downwards and produced by the healing

up of wedge-shaped excisions did not disturb the normal course of the lateral line. If a tail or the anterior half of an embryo is implanted behind the place at which the vagus nerve takes its rise the lateral line coming from the front part continues to grow in a straight line. If an embryo is constructed of three transverse pieces, the middle piece being turned the wrong way about, the lateral line is able to grow through this middle piece. But this power of penetration is confined to certain stages.

Several investigators have studied the results of removing parts of the nervous system. Schaper (1897) removed the brain from larvae of *Rana esculenta* [XV, 15] by a horizontal section and yet observed a continued development of the larva [XV, 15*a*], which only lacked the parts directly removed. Harrison (1903, 1904) removed the dorsal half of the spinal cord from embryos of *Rana silvestris* and *R. palustris*, and found that the muscular system including the myotomes and hind legs (H. L. Langnecker's experiments) was developed as in normal embryos [XV, 16*a*]. If the appearance of the Schwann cells was prevented in the embryos of *Rana esculenta* by an early excision of the spinal cord [XV, 16] the axis cylinders of the motor nerves developed notwithstanding [XV, 16*a*], but consisted only of naked fibres which could be traced into the ventral musculature of the head and tail (Harrison, 1904). If on the other hand the rudiments of the motor nuclei are removed by a longitudinal excision of the lower half of the detached medullary tube [XV, 17] the motor nerves do not grow although Schwann's sheaths are formed enveloping the sensory nerve fibres [XV, 17*a*].

Babák (1905) removed the brain from various Anura (*Rana esculenta*, *Bufo*) as far as the medulla oblongata and found that the metamorphosis was retarded, the gills and tail persisting longer than in intact forms. "Most frequently, indeed almost regularly, the reduction of the gills and tail takes place separately, when the operation to the brain is performed some days before the appearance of the fore limbs, a moment which may easily be determined by outward signs."

But in the metamorphosis of frogs independent differentiations of considerable importance occur; for instance, according to Braus (1906) the presence of the fore limb is not necessary in the case of *Bombinator* larvae for the formation of the perforation hole; in typical development the presence of the limb leads at the time of metamorphosis to the loosening of the gill sac through which it emerges.

(c) *Amniota.*

That few experiments have been made on the mechanism of development among the *Amniota* is doubtless due in large measure to the inaccessibility of the embryos. In most reptiles, in birds, and among mammals in the duckbills, the eggs are very rich in yolk and surrounded by a resistant shell; in the case of some reptiles, such as the slow worm and common adder, and in that of the mammals with the exception of the duckbills, no eggs are laid, but the embryos develop within the maternal organism and only leave the egg envelope at birth.

The difficulties presented to the experimenter by the reproductive methods of the amniotes are counterbalanced by only one advantage: the ease with which the egg of one animal belonging to this group, the common fowl, may be obtained. A number of experiments have been made on this egg.

As early as 1877 Dareste (cf. 1891) employed hens' eggs for the artificial production of monstrosities, but these experiments afford no clue as to the agencies at work.

By varnishing a strip of the shell situated above the germinal disc Gerlach (1882) succeeded in some cases in obtaining a double formation of the anterior half of the embryo, i.e. in demonstrating a greater potency in the anterior embryonic cells than would pertain to them according to their prospective significance. Similar experiments are to be found in Mitrophanow's work (1900), though their results are less favourable to this conclusion.

Assheton (1896), Peebles (1898, 1903—1904) and Kopsch (1902) removed a small piece of the shell above the germinal disc, and having inflicted certain injuries closed the hole again with a piece of shell, using the egg membrane as binder. It appeared that the "primitive streak" does not form the anterior sections of the body (head, neck, heart region?), but that these arose anterior to a mark made by pricking just in front of the primitive groove [XV, 19].

If certain regions of the germinal disc are destroyed the corresponding parts of the body do not develop while the others differentiate further. A high capacity of self-differentiation in the tissues of the chick embryo is also indicated by Jan Tur's (1904) experiments on the influence of radium on hens' eggs; 80 hens' eggs were exposed for from 24 to 70 hours to radium chloride and all showed deformation

of such a kind that the central parts and the rest of the ectoderm suffered while the endoderm continued to develop.

Summing up the results which have been obtained by experiment with regard to the localisation of the several embryonic rudiments in the (fertilised) egg, we may state that:

In different zones of the egg different (chemical) substances are present which normally cause the differentiation of the various organs in their proper regions. If these substances become distributed elsewhere by cleavage, gastrulation and the later processes of differential growth they produce the same effect in their new positions. If a reduction of the egg material, unaccompanied by a rearrangement of the various substances, is made, the prospective significance of the blastomeres leads to a self-differentiation of the reduced portions whereby half, quarter and one-eighth.....forms arise; if on the other hand a rearrangement of the egg content is possible in such a way that all the substances occupy the same positions with regard to each other as in the intact egg, perfect forms arise on a proportionately diminished scale; the prospective potency of such blastomeres is greater than their prospective significance.

CHAPTER IX.

INFLUENCE OF EXTERNAL FACTORS.

1. Chemical agents.

(*a*) *Necessity of certain agents.*

We have seen that the differentiation of animal embryos may be referred to the formative activity of chemical substances which are already present in the eggs. At the same time the eggs are supposed to develop in their normal environment. If eggs are exposed to abnormal environments it appears that many species, if they retain their vitality at all, are quite independent of the chemical composition of the outer medium. Thus *Ascaris* develops in Flemming's fluid (Bataillon 1901), *Fundulus* (Loeb 1901—1902) in distilled water. Such eggs possess all the necessary materials, not only for the determination of the form of various organs, but also for assimilation and the maintenance of a certain physical pressure (osmotic "salts"); and these materials are present in a quantity sufficient for complete embryonic development. Probably such eggs exchange gases and salts with the surrounding medium only to a trifling extent, even under normal conditions. Even for these eggs a certain amount of oxygen is necessary both for supporting life and for development. Here we will merely refer to the experiments on this subject made by Dareste (1891), Koch (1884) and Mitrophanow (1900) on hens' eggs, by Morgan (1905) and Schultze (1899) on frogs' eggs, by Loeb on fish (1894) and sea urchin eggs (1895, 1906). Most eggs, for example those of the sea urchin, require for their best development a definite composition of the external medium. For sea urchin eggs the optimum of development coincides almost exactly with the distribution of chemical materials in the normal environment, that is sea water (Herbst 1897, 1904), yet a somewhat greater alkalinity of the water seems to favour at least the early stages of development: Loeb

(1898) found that the development (of *Arbacia pustulosa*) was accelerated by the addition of a certain quantity of sodium hydrate (NaOH) to ordinary sea water. The size of the larvae can also be increased.

Herbst prepared artificial sea water in order to have the elimination and substitution of the constituents under control. In accordance with an analysis made by Forchammer of a sample of water drawn between Sardinia and Naples the following constituents were dissolved in 1000 parts of distilled water: 30 parts by weight of common salt (NaCl), 0·7 potassium chloride (KCl), 5 magnesium chloride ($MgCl_2$), 2·6 magnesium sulphate ($MgSO_4$), 1 sulphate of lime ($CaSO_4$): then to 1000 cm.³ a pinch of subphosphate of lime ($CaHPO_4$) was added and after 15 hours filtered off. Finally a pinch of precipitated carbonate of lime was mixed in, and carbonic acid gas passed through the solution for from ½ to 1½ hours; the solution was then allowed to stand 12 hours in a closed vessel, filtered, shaken up with air and preserved in shallow glass vessels covered with wet filtering paper for from 24 to 48 hours. In order to test whether the presence of any particular constituent was necessary, the same method of preparation was employed, but instead of the corresponding combination another one equimolecular (i.e. isotonic) with it was chosen which did not contain the substance to be tested. It appeared that from the beginning of the development chlorine (Cl), hydroxyl ions (OH), sodium (Na), potassium (K), and lime (Ca) must be present, while sulphate (SO_4), carbonate (CO_3) and magnesium need only be supplied to the larva in the later stages; phosphorus (P) and iron (Fe) do not seem to be absolutely necessary to development, although the former is always present in natural sea water.

To eliminate the chlorine common salt (NaCl) was replaced by sodium formate (3·07% HCOONa equimol. 3% NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$), and by sulphates (0·12% K_2SO_4 + 0·4% $MgSO_4$); in these cases the cleavage did not complete its course.

The sodium was eliminated by substituting magnesium chloride ($MgCl_2Ca$ 3%) for common salt (NaCl); the cleavage was abnormal and furnished at most 48 cells. That the unfavourable result could not be referred to the increase of the magnesium chloride was shown by a parallel experiment in which 1·34% magnesium chloride was added to sea water containing NaCl; in spite of the increase of osmotic pressure thus produced gastrulation occurred.

The antagonistic action of sodium (cell loosening) and potassium (cell cementing) has already been discussed with reference to the causes which determine the coherence of the cleavage cells (Ch. VI). Potassium and magnesium may simply be eliminated without any important change of concentration.

Herbst placed sea urchin eggs in water devoid of potassium and found that *Echinus* perished immediately; *Sphaerechinus* segmented but only produced small and turbid blastulae [XVI, 2]; this is referred to a deficient absorption of water since potassium is conducive to absorption. Loeb has seen frogs' muscles increase in weight by 45·7% within 18 hours when placed in a solution of KCl, while the increase in an equimolecular solution of common salt (NaCl) only amounted to 6%. Magnesium is necessary for the differentiation of the regions of the alimentary canal (and for ciliary movement).

Sulphur is indispensable in normal development up to and beyond the blastula and gastrula stage [XVI, 1]. The sulphates (SO_4) which are active in sea water are of special importance for the complete development and direction of the intestine, for the formation of pigment and the structure of the larval form (position of the cilia rings, bilaterality). The sulphate serves in addition to prevent the hypertrophy of the tuft of cilia in the *Echinus* larva, a process strongly promoted by calcium, indeed the effect of the calcium may be so great that more than a half of the larva is covered with long cilia [XVI, 3].

Finally sulphate (SO_4) and magnesium contribute to the formation of the pluteus skeleton which consists chiefly of carbonates of lime ($CaCO_3$). In an environment devoid of SO_4 the lime secreting cells which are otherwise arranged bilaterally right and left of the archenteron, in the form of two triradiate stars at some distance from the anus, are deposited in an abnormal and irregular manner. They remain more or less in the neighbourhood of their place of origin so that they closely surround the archenteron instead of lying at a distance from it and adjoining the ectoderm [XVI, 4]. "Still more striking is the disturbance of the normal bilateral arrangement of the skeletogenous cells when the larvae are removed from water devoid of SO_4 and placed in that containing SO_4. The lime forming cells then migrate from the archenteron and arrange themselves (in optical section) on the periphery beneath the ectoderm in the form of more than two triradiate stars."

On the necessity of lime for the development of the pluteus

skeleton we have the older experiments of Pouchet and Chabry. Eggs from fifteen ovaries were examined chemically and found to yield no more than a trace of lime; since the larvae always perished when placed in artificial sea water devoid of Ca these investigators attempted to precipitate the Ca in natural sea water by means of oxalate of ammonium, potassium or sodium; only in the case of the last precipitation was the pluteus stage reached at all; the plutei were however without skeleton and of spherical form [XVI, 5]. According to Herbst calcium carbonate is indispensable for the formation of the calcareous spicules of the pluteus (*Sphaerechinus*, *Echinus*), even when sulphate of lime or chloride of lime is present. From mixtures devoid of $CaCO_3$ the blastulae have a crumpled appearance, especially in the case of *Sphaerechinus*. The later the stage at which the larvae are placed in the solution devoid of $CaCO_3$ the rounder the form of the pluteus, and the skeleton is never formed; indeed in the case of fully developed larvae it actually disappeared in the same solutions which permitted larvae of the same sea urchin (*Echinus*), obtained from eggs immersed in the solution since the time of impregnation, to produce rudimentary skeletons, which they did not subsequently lose.

O. Maas (1904[1]) has made similar experiments on calcareous sponges, in particular *Sycandra setosa* [XVI, 8—10], rearing them in solutions devoid of carbonate of lime, and has arrived at results precisely similar to those of the earlier observers in the case of sea urchins. Here too the development of the skeleton, which consists of spicules of calcite, is dependent on this lime salt, while the presence of other lime salts (SO_4, Ca, etc.) is not sufficient. In this case also the development of the utricular body form is dependent on the development of the skeleton, and the cells destined to form the skeleton attempt to produce needles of plasm; such a foundation also appears to be normally present as a sort of net in the spicules since they are attacked by sodium hydrate in such a way that the crystalline substance which had originally secreted uniformly, now falls into a number of smaller crystals (1904[2]).

The sponge embryos devoid of $CaCO_3$ remain spherical [XVI, 11], or fall into plate-like groups of cells, which may be arranged around several cavities, but perish if the normal body form is not developed. In like manner the cells which normally form a fibrous calcareous root tuft at one pole of the sponge begin to produce delicate webs like those of a spider which appear in several places lying abnormally;

the root tuft itself naturally fails to appear. These irregularities are determined by the absence of the calcareous needles and not by the direct action on the plasm of the altered composition of the water, which is devoid of $CaCO_3$. This is confirmed by Maas' (1904[2]) parallel experiments with siliceous sponges (*Gellius varians*, and a species of *Reniera*) which develop their siliceous spicules and normal form (with flagellated chambers and oscular tube). Maas demonstrated by the following method of preparing sea water that the larvae of these siliceous sponges already possess a sufficient supply of silica (SiO_2) to form the skeleton. The method consisted in evaporating and then dissolving the solid constituents in an equal quantity of water, a process in which the carbonate of lime (CO_3Ca) and the silica become indissolubly precipitated and are not again dissolved.

In more recent experiments Maas (1906) employed artificial sea water prepared according to Herbst's process. "The continued effect of water devoid of lime affords a means of completely separating the granular cells [XVI, 8, 9], so that finally a 'blastula' remains which consists solely of flagellate cells. By removing such isolated halves of an original amphiblastula, now reduced to flagellate cells, into normal sea water we can obtain some idea of the nature of the two halves of the larva and the potency of the cells. The ciliated blastulae mentioned above, which correspond to the anterior half, may remain active for a considerable time, even longer than a week. Alteration of the flagellate cells, and transformation into granular cells, such as has been described by Minchin in the normal larva of *Ascon* and by me (Maas) in *Oscarella* does not occur. Such larvae never succeed in attaching themselves; they thus behave precisely like the animal parts of echinoid larvae obtained by Driesch." "On the other hand halves of the larvae consisting of granular cells, i.e. little heaps of granular cells left on the ground from which the flagellate cells have swum away, are able when placed in normal water to attach themselves and produce a gastric cavity; but in these cases it is not quite certain whether we have to do with purely 'vegetative' halves.

"If amphiblastula larvae which are altered to a less degree (i.e. larvae in which there are still sufficient granular cells, and in which loosening of the cell connexion is scarcely perceptible) be brought from water completely devoid of lime into normal sea water, recovery is still possible, and a little sponge capable of performing its functions with pores, osculum, and spicules may be produced."

If the lime is subsequently withdrawn, the calcareous needles of

the early live stages are more quickly disintegrated than those of later stages, or of dead sponges. It is possible that the more powerful separation of carbonic acid in young animals and the assimilative processes of the formative cells help to produce this effect.

How far the skeleton forming substances can be replaced by others in one and the same form will be investigated by Maas in further experiments, similar to those which have already been made by Herbst in continuation of his studies of the sea urchin.

Herbst (1901) arrived at the conclusion that sulphates can only be replaced by combinations of sulphur when these also give rise to sulphates. Selenates and tellurates cannot be employed in the place of sulphates, although selenium and tellurium are the elements which most closely resemble sulphur. If chloride be replaced by an equimolecular quantity of bromide (3°/₀ NaCl by 5·28°/₀ NaBr, etc.) a very meagre development may take place; equimolecular iodine cannot replace chloride. This also holds for polyps of *Tubularia mesembryanthemum* and eggs of the fish *Labrax lupus*. Calcium cannot be replaced by magnesium, strontium (Sr) or barium (Ba) in the formation of the skeleton, either in the case of sea urchins or vertebrates. The effect is interesting when potassium chloride is replaced by rubidium chloride and caesium chloride. If equimolecular quantities (0·08°/₀ KCl mol. = 0·13°/₀, RbCl mol. = 0·18°/₀ CsCl) are employed we do not obtain the most favourable result in any given case because the active strength of equimolecular solutions of KCl, RbCl, and CsCl increases with the rising molecular weight of these constituents. The optimum conditions are also different for the different processes of development; when the concentration most favourable for increase of size and the normal clear appearance of the larvae is reached, the concentration for the separation of the framework is already exceeded, so that in these solutions the plutei, which are otherwise very well developed only possess a rudimentary skeleton.

(*b*) *Harmful elements.*

We speak of a poisonous effect when the harmful alteration in the environment consists, not in the withdrawal of necessary materials or their insufficient supply, but in the addition of substances which normally are either not present or are hindered in their action by others of opposite effect. Loeb in particular has shown in a number of publications (1901[3], 1902[2], Loeb and Gies 1902) that even the substances which occur in the normal environment of a species may

develop a harmful action, as soon as other substances are absent, which are normally necessary for "physiological equilibrium."

"If sea urchin eggs, freshly fertilised, are placed in a solution of $\frac{5}{8}n$ NaCl the eggs do not develop. As a general rule they do not even segment. If a small quantity of a bivalent kation, e.g. calcium, is now added, it is unable to neutralise the poisonous action of the sodium ions. No swimming embryo (blastula) is developed although the eggs are able to begin the process of cleavage. The action of the calcium ions in destroying the poisonous effect of the sodium ions at once appears when a second body ('mid-body'), namely a small quantity of potassium ions, is added. The eggs then form not only swimming embryos (blastulae and gastrulae) but, other conditions being equal, also attain to the full duration of life of larvae bred in normal sea water (about 8—10 days in my [Loeb's] experiments)."

If the potassium ions are alone added (without the calcium ions) the cleavage continues for some hours, indeed, under circumstances particularly favourable, even the blastula stage may be reached, but after this development comes to a standstill (1901, p. 75). The influence of potassium alone is therefore trifling.

Generally speaking the valency of the kation appears to play an important part. Loeb's theories cannot here be discussed further since they would be incomprehensible without much detailed information on the conditions necessary for life, and this would be out of place in a work on embryology.

The poisonous effects of substances which are not normally present in the environment may be of interest either because they demonstrate the importance of the chemical composition, even in liquids of equal concentration, or because they produce particular formative effects. For the former case compare for example the influence of sugar solutions on frogs' eggs, according to Gurwitsch (1896), and Jenkinson (1906), and for the latter the influence of lithium which in the case of sea urchin eggs produces "exogastrulation" [XVI, 6] according to Herbst (1895), in frogs' eggs radially symmetrical gastrulae, according to Gurwitsch, and various deformations according to Morgan; boric acid produces "telescope nose" in frogs' embryos, according to Roux (1889, cf. 1895, II, p. 887). Finally the chemical side of the question is interesting because a regular connexion may be shown to exist between the extent of the poisonous action and the chemical composition of the poisonous series employed.

Thus, according to Herbst's experiments with sea urchin eggs (1893), the action of one and the same salt decreases in the case of the salts of monobasic acids with an *increasing* molecular weight. But lithium salts have a stronger action than potassium salts and these a somewhat stronger action than analogous combinations of sodium in solutions of equal concentration.

For the production by means of lithium of the "exogastrulae" mentioned above, lithium chloride (molecular weight 42½) is most suitable on account of its stronger action; the action of lithium bromide is weaker (87 M.) and that of lithium iodide (134 M.) the weakest of all. This agrees with the rule given above.

According to Bataillon (1901) neither the solutions of cane sugar nor those of lithium or other salts produce their effect as the result of a different chemical action but simply in consequence of the different molecular pressure of solutions of equal concentration. Solutions rendered isotonic are said always to produce the same effect on frogs' eggs.

It is certain that the forms produced by lithium and the influence of other salts can be obtained in more than one way; for example, exogastrulation can be obtained by means of an injurious degree of warmth (Driesch 1892).

Féré (1894[2,3]) investigated the poisonous qualities of alcohols by injecting them into hens' eggs; he found that in the series ethylalcohol, methylalcohol, propylalcohol, butylalcohol, amylalcohol, each member is more poisonous than that preceding it. The iso-alcohols were more poisonous than other corresponding alcohols. Aceton had the same effect as ethylalcohol. Quite recently Fühner (1904) has obtained even more exact data for sea urchin eggs (*Strongylocentrotus lividus*).

"In the homologous series of the monovalent primary alcohols the activity of the normal members (members with a single carbon chain) increased by a constant amount. Each member is three times as active as the preceding. The members with a branched carbon chain and the secondary alcohols are less active than the primary."

O. and R. Hertwig (1887) have experimented on sea urchin eggs in various stages of development in order to determine the effect of various violent poisons, nicotine, morphium, strychnine, chloral hydrate, chloroform, cocaine, quinine; but no definite rule seems to have been observed, with regard to the chemical composition of the poisons.

2. Moisture.

(*a*) *Necessity of moisture.*

For the normal development of animal eggs a certain amount of moisture is necessary but this appears to vary within very wide limits. Cases which exhibit this variation occur in particular among the Amphibia. P. Kammerer has made extensive investigations on this subject, the chief importance of which lies however in the domain of the formation of species. Here we will only briefly cite two of his results.

"Embryos of *Salamander atra*, which are removed by operation from the uterus of the maternal parent may be reared in water from the 'second stage' after leaving the egg envelope" (1904).

"If we mature *Alytes* spawn in its normal environment on land, we may observe that the larvae which emerge and crowd into the water require a period of development many times longer than that of the other anura; and *Hyla* spawn matured on land gives larvae which require a year for their development instead of a few weeks. But if we bring *Hyla* spawn to maturity in its normal environment in water the larvae born in May are metamorphosed in August of the same year; and the same occurs when *Alytes* spawn develops abnormally in the water; the young toads are developed from the larvae in an equally short time" (1906).

(*b*) *Harmful effects of moisture.*

On the other hand when Kammerer (1904, p. 189 with bibliography) placed in water fertilised eggs of *Salamandra atra*, which had been removed from the oviduct or uterus he did not succeed in obtaining embryos from them, a result in contrast to his success with *S. maculosa* (p. 237). In the case of *atra* the cleavage began its course but the eggs soon swelled up and after some weeks perished.

3. Density.

(*a*) *Necessary degree of density.*

When dealing with fertilisation, nuclear division and the influence of the chemical environment we have frequently pointed out the importance of observing the concentration of the solution. As a

general rule it appeared that two solutions do not produce the same effect when they contain the same percentage of a given substance (specific density) but when they exert the same molecular pressure ("isotony") (cf. in particular Bataillon, Herbst, Jenkinson, Loeb).

(*b*) *Harmful degree of density.*

The injurious effects of rarefied air have been investigated by Giacomini (1899) who has made experiments with hens' eggs.

4. Mechanical agents (pressure, operation).

(*a*) *Necessary mechanical agents.*

Experiments with blastomeres derived from forms in which the plasm is capable of regulation show that the manner in which a blastomere will behave depends on whether it is in complete contact with its opposite blastomere (half form) or is separate from it (perfect form). From this it may be inferred that the pressure of the cells against each other normally plays a certain part in the formation of the organs. The same fact may be deduced from the equal development which takes place on all sides of the cell when the cell union is loosened by the withdrawal of lime (cf. Herbst 1899, Wilson 1904[2,3]).

(*b*) *Harmful mechanical agents.*

In the unfertilised egg of the sea anemone increase of pressure in certain directions leads, according to King (1906), to the retention of the polar bodies. The alteration of the course of cleavage by pressure or other mechanical means has been treated in detail above (chapter VIII) and the facts need not be recapitulated here. By making incisions in hens' eggs Schrote obtained monstrous forms, for example forms possessing two hearts, as early as 1862.

5. Force of gravity.

(*a*) *Necessity of force of gravity.*

We have seen that according to Roux' experiments, which have been confirmed by a number of other investigators (Moszkowski 1902, Morgan 1904[1]), the development of frogs' eggs is independent of any

particular position with regard to the force of gravity. This is not contradicted by the fact that if the eggs are inverted abnormal forms are produced; this effect, as shown by the experiments of Born, Schultze, Wetzel and Morgan, is a result of the rearrangement of substances of different weight which disturb the normal course of development.

(*b*) *Harmful effects of the force of gravity.*

While many eggs, for example those of the praying mantis, can assume any position with regard to the centre of gravity during the whole of the period of development, without experiencing the slightest disturbance, others are more sensitive to inversion and only produced crippled larvae, e.g. those of the water beetle (*Hydrophilus*), according to Megušar (1906). These are normally maintained in an upright position by a peculiar mast of the boatlike cocoon, which swims in the water, while the cocoon of the praying mantis settles in any position on stones, stalks and bushes. Centrifugal force is also injurious if in such strength as to cause the egg substances to lie in the order of their weight, i.e. if it disturbs the relative position necessary for differentiation (cf. O. Hertwig 1897, Lillie 1906, Morgan 1906).

6. Electricity (and magnetism).

(*a*) *Necessity of electricity.*

In the case of plants experiments have been made by placing wire caps over them to exclude the atmospheric electricity, and these have shown the usefulness of this force to the plants, but I have not been able to find data of similar experiments in the case of animals.

(*b*) *Harmful effects of electricity.*

The appearance of "Framboisia" in frogs' eggs when placed under the influence of the electric current has been pointed out in connexion with Roux' experiments on the arrangement of the cleavage cells (chapter VI). Rossi (1896) in the case of the eggs of *Salamandra perspicillata* obtained no effects from the electric current other than would be produced by injurious factors, namely faulty segmentation of the white pole and irregular cleavage.

Maggiorini (1879) made experiments on the influence of magnetism on the fertilised bird egg. On fixing a magnet beneath a nesting basket or box containing pigeon, hen, or canary eggs, these at first showed a check in development and later an acceleration of the hatching process resulting in young of high vitality, but of diminutive size and with imperfect feathering of the neck. These remarkable observations however have not been subsequently confirmed.

7. Light and other radiant energy.

(*a*) *Necessity of these factors.*

In many animals development takes place under such conditions that the participation of light appears to be normally excluded from the beginning. This occurs with animals which live in caves or the deep sea, with those in which the egg envelope is completely impervious to light and with those in which the embryonic development takes place within an opaque maternal organism.

In many animals want of light appears to protract the hatching process, but this seems to be due rather to the absence of the movement stimulus which causes the breaking of the egg envelope than to retardation of the process of differentiation. Thus in the praying mantis (*Sphodromantis bioculata*) a considerable retardation occurs when the cocoon is placed in the dark after the escape of the first larva although normally all the young leave the egg on the same day (Przibram 1906). The whole post-embryonic development of this animal may also be carried out in the dark without producing any effect on the colouring. According to Yung (1878) complete darkness may retard the development of frog larvae, but does not prevent it.

(*b*) *Harmful effects of light and other radiant energy.*

Schnetzler (1874) states that frog larvae placed under a green glass are retarded in development and this is also maintained by Yung (1878) in the case of red. My own experiments with *Sphodromantis* (1906) showed that green, red and yellow glasses had an unfavourable influence, but this may have been due to the heating of the metal cages employed, since the red glass was less transparent and may not have allowed so many heat rays to pass through. In

any case blue and violet glasses are more favourable than red, yellow or green and also than white, as is shown by Yung's experiments on the eggs of *Rana temporaria, esculenta, Salmo trutta* (trout) and *Limnaea stagnalis* (pond snail). Yung found violet, blue, yellow and white, in a decreasing series, more favourable, and red and green more injurious than darkness. The normal white light thus seems to exercise a certain injurious effect. Röntgen and radium rays are harmful to the development of eggs. These experiments have little interest for zoology with the exception of the works of Tur already quoted and a very recent article by Bardeen. We may refer to the bibliography in Bardeen: "Abnormal development of Toad ova fertilized by Spermatozoa exposed to the Roentgen 'rays'" (*J. of Exp. Z.* IV, no. 1, 1—44, 1907).

8. Warmth.

(*a*) *Necessity of warmth.*

For all animal eggs a certain degree of warmth is requisite if they are to develop at all, but this temperature is very different for different species. While trout eggs develop with a temperature a few degrees above freezing point, I found that in the case of the praying mantis development is impossible with the temperature below 17° C. In general development is accelerated with a rising temperature, indeed it becomes two to three times as rapid when the temperature rises 10° C. (Van't' Hoff's rule). This law, which is true of chemical reactions and also of other inorganic processes, was confirmed by O. Hertwig (1896) in the case of frogs' eggs, and by Peter (1905) in that of sea urchin eggs. With regard to organic life the law holds after the development of the egg and thus demands a closer investigation in connexion with the conditions of life in general. According to Galloway (1900) the favourable action of warmth in Amphibian eggs consists in its power of increasing the absorption of water.

(*b*) *Injurious effects of heat.*

With a rising temperature a degree of heat is finally reached which destroys the vital process completely and with it the embryonic development. On the influence of such injurious temperatures as well as those which are too low compare: Dareste

(1891) for the chicken, H. D. King (1903) for the toad (*Bufo lentiginosus*), O. and R. Hertwig (1887), O. Hertwig 1891, Driesch (1892) and others for sea urchins.

Driesch found that if blastulae of *Sphaerechinus granularis* were fertilised in a temperature of 15° C. and placed 18—27 hours later in an oven at (30° C.), with a sufficient quantity of water, they exhibited after 18 hours a small outgrowth, the rudiment of the archenteron, which had grown in the wrong direction, but they swam round in an active manner [XVI, 7]. The extruded gut may become differentiated into three parts, but then shrinks and may disappear completely (Anenteria).

If we consider the influence which external conditions exercise upon the embryology of animals [*as compared with the developmental forces inherent in the eggs themselves*] *we find that for the normal course of embryonic development a certain chemical composition together with a degree of moisture and concentration are requisite in the environment, and that warmth, light, and to a certain extent the force of gravity are also necessary; but the external conditions may vary within wide limits without altering the typical development of the animal form. Natural conditions are not always the most favourable; we may recall the influence of alkali in sea-water and of violet and blue light as opposed to white.*

The influence of external factors in animal embryology is of minor importance as compared with the forces of development within the egg, so that the latter may be described in general as constituting an almost perfect process of self-differentiation, as understood by Roux.

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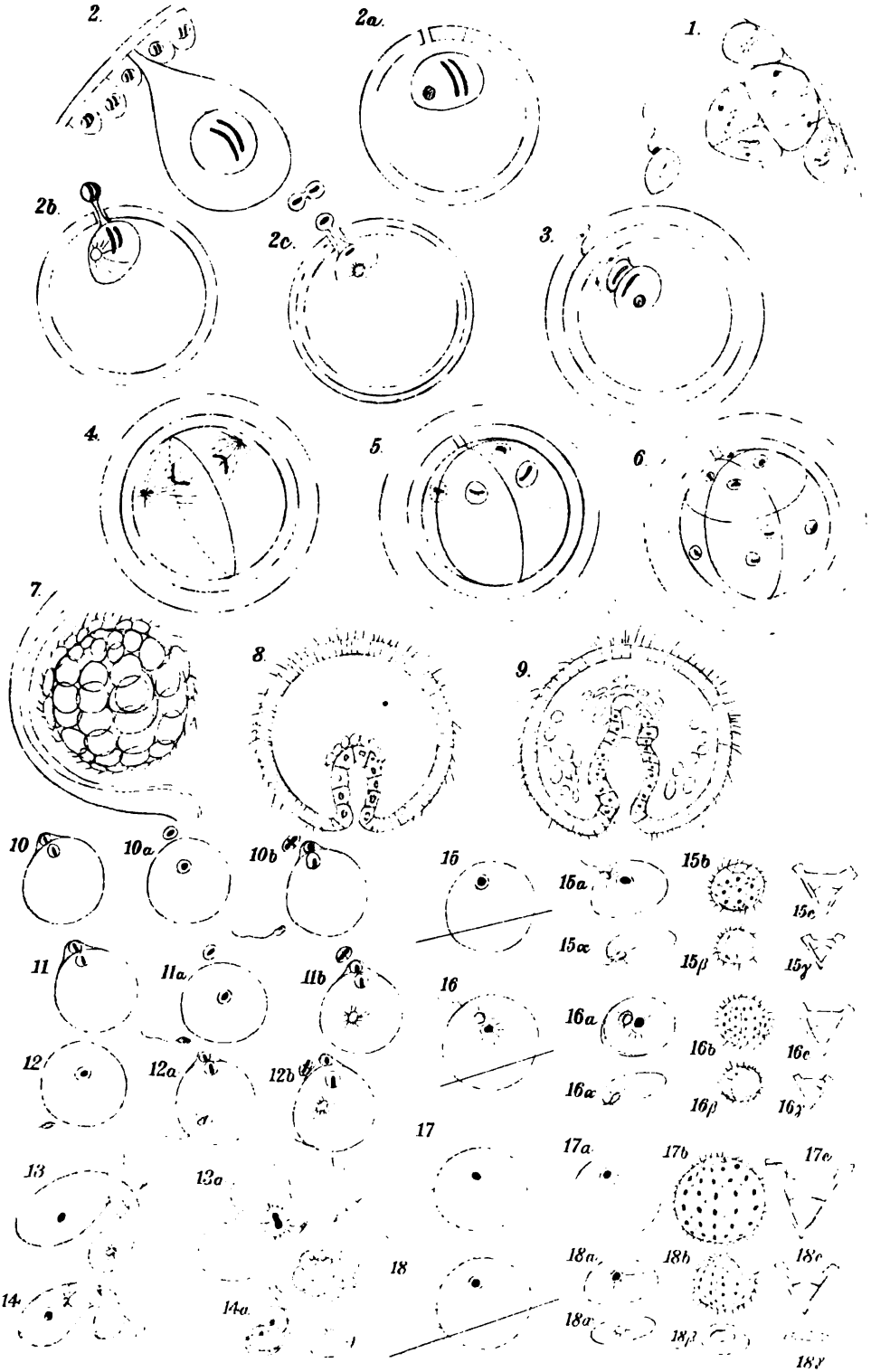

PLATE II.

STRUCTURE OF THE EGG OF INVERTEBRATES (WITHOUT CHORDA).

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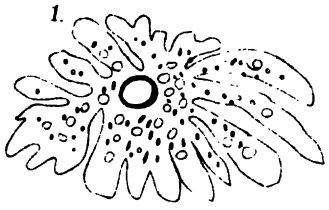
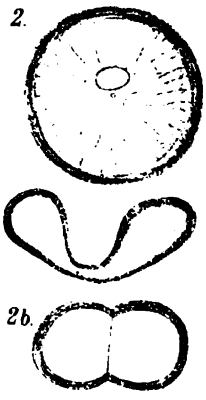
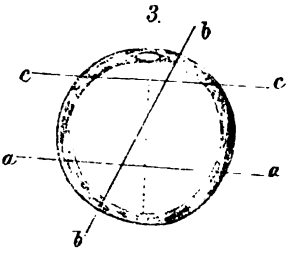
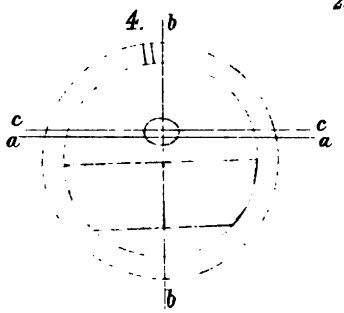
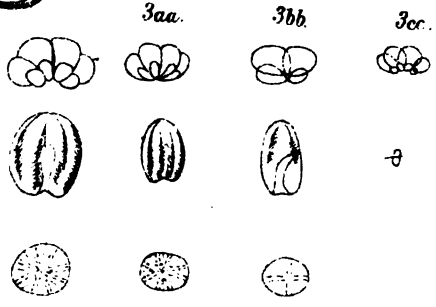
Fig. 5. Egg of *Myxostoma*, after Driesch.
Fig. 5 *a*. Two-cell stage of same.
Fig. 6. Eggs of *Mantis religiosa* (praying cricket) in the cocoon (section).
Fig. 6 *a*. Single egg of an insect, after Korschelt.
Fig. 7. Egg of *Ilyanassa obsoleta* (snail), after Crampton.
Fig. 7 *a*. Constriction and 7 *b* two-cell stage, after Crampton.
Fig. 8. Egg of *Dentalium entale* (Scaphopod), after Wilson.
Fig. 8 *a*, *b*, *c*. Development when cut up along lines *aa*, *bb*, *cc*.
Fig. 9. Egg of Loligo (squid), after Watasé.
Seen from the side and above (90).
Fig. 10. Egg of *Cerebratulus lacteus* (nemertine), after Wilson.
Fig. 10 *a*, *b*, *c*. Development when cut up along lines *qa*, *bb*, *cc*.

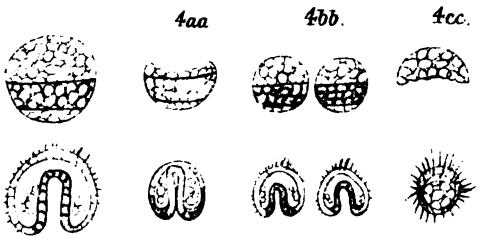


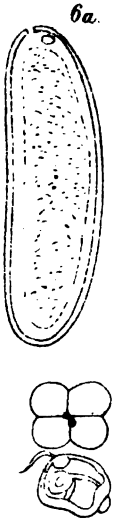


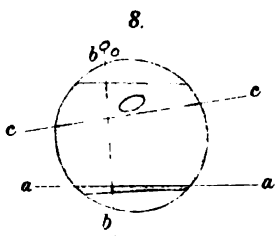


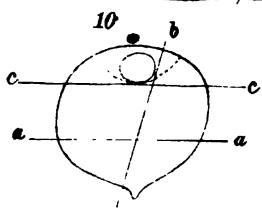


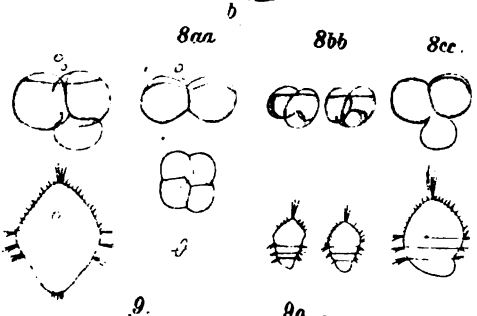


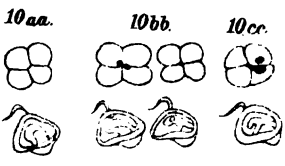


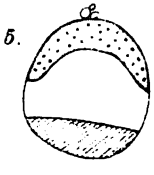


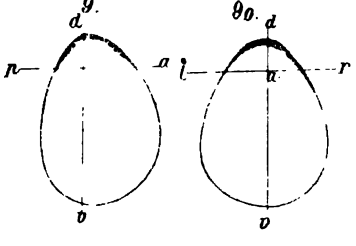


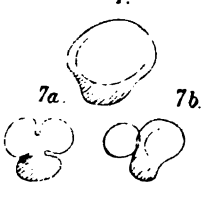












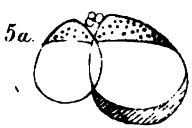


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PLATE III.

STRUCTURE OF THE EGG IN VERTEBRATES AND OTHER CHORDONIA.

Fig. 1. Egg of *Amphioxus lanceolatus* (lancet fish), after Delage.
Fig. 2. Egg of *Petromyzon fluviatilis* (lamprey), after Herfort.
Fig. 3. Egg of *Rana fusca* (brown frog), after Korschelt and Heider.
Fig. 3 *a*. Same seen from above (without envelope).
Fig. 4. Egg of *Rana esculenta* (water frog), after Korschelt and Heider.
Fig. 4 *a*. Same seen from above (without envelope).
Fig. 5. Frog's egg in position of constraint (section, two stages), after Born.
Fig. 5 *a*. Same, seen whole, from the side.
Fig. 6. Diagram showing rotatory structure of an egg.
Fig. 7. Cleavage of egg in *Rana temporaria* (brown frog), after Ziegler.
Fig. 7 *a*. Cleavage of same egg when centrifugalised, after Hertwig.
Fig. 8. Egg of *Gallus domesticus* (common fowl) opened, after Waldeyer.
Fig. 9. Egg of *Rana fusca* (brown frog), Diagrammatic representation of the typical (═) and (::::) atypical pigment path.
Fig. 9 *a*. Result of the first cleavage with typical pigment path.
Fig. 9 *b*. Same egg, first furrow turned to the front.
Fig. 10 *a*. Result of the first cleavage with atypical pigment path.
Fig. 10 *b*. Same egg, first furrow turned to the front.
Fig. 11. Egg of *Rana fusca* (brown frog) reconstructed from sections parallel to the first furrow, after Roux.
Fig. 11 *b*. Same egg, seen from above, after Roux.
Fig. 12. Egg of *Rana fusca*. Normal, with the nuclear spindle of the first furrow. Here and in the following figures of this plate the little cross indicates the point at which the spermatozoon enters, the rhombic figure marks the position of the first spindle of nuclear division, the little circle the resting nucleus.
Fig. 12 *a*. Same after completion of the first furrow.
Fig. 13. Egg compressed between horizontal glass plates, after Born.
Fig. 13 *a*. Same after completion of the first furrow.
Fig. 14. Egg compressed between vertical glass plates, after Born. Showing edges and surface of glass plates.
Fig. 14 *a*. Same after completion of the first furrow, after Born.
Fig. 15. Eggs introduced by suction into narrow glass tubes, after Roux. View of the meridian of sperm entrance, and a view at right angles to the first.
Fig. 15 *a*. Same after completion of the first furrow.
Fig. 16. Eggs introduced by suction into broad glass tubes, after Roux. 1st possible position of the first spindle of nuclear division.
Fig. 16 *a*. Result of same after completion of the first furrow.
Fig. 17. Eggs introduced by suction into broad glass tubes, after Roux. 2nd possible position of the first spindle of nuclear division.
Fig. 17 *a*. Result of same after completion of the first furrow.

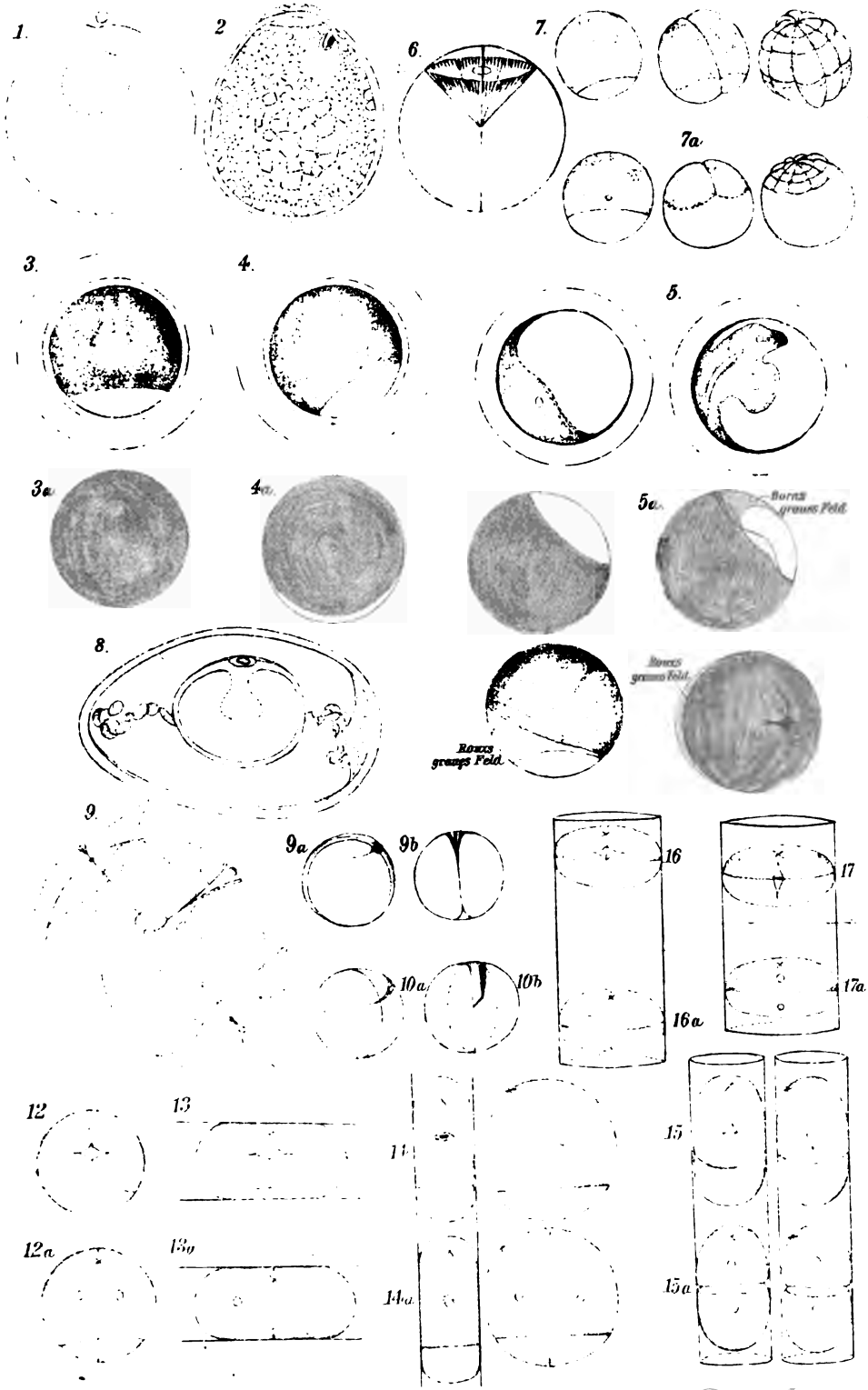

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PLATE IV.

MITOTIC CELL DIVISION.

1. *Migration of the nucleus.*

Fig. 1. Egg of *Ascaris megalocephala* (horse Ascaris), after Boveri. ♂ denotes sperm nucleus, ♀ egg nucleus, *p K* polar bodies.
Fig. 2. Same, preparation for the first spindle of nuclear division, after Boveri.
Fig. 3. Same, formation of the first spindle of nuclear division, after Boveri.
Fig. 4. Same, cleavage of the first spindle of nuclear division, after Boveri.
Fig. 5. Same, incision of the first furrow, after Boveri.
Fig. 6. Same, two-cell stage, after Zur Strassen.
Fig. 7. Same, preparation for the four-cell stage, after Zur Strassen.
Fig. 8. Same, four-cell stage, T-form, after Zur Strassen.
Fig. 9. Same, four-cell stage, assumption of rhomboid form, after Zur Strassen.
Fig. 10. Same, four-cell stage, rhomboid form, after Zur Strassen.
Fig. 11. Diagram of the two-cell stage, after Zur Strassen.
Fig. 12. Diagram of centrosome migration during division of a cell situated in a bent epithelial tissue, after Zur Strassen.
Fig. 13. Diagram of centrosome migration during passage of two-cell to four-cell stage, after Zur Strassen.
Fig. 14. Diagram of centrosome migration during division of a cell situated in a straight epithelial tissue, after Zur Strassen.
Fig. 15. Embryo in median section, after Zur Strassen.
Fig. 16. Diagrammatic representation of the conditions of polarity in eggs with "rotatory structure," first furrow.
Fig. 16 *a*. Egg with rotatory structure, second furrow, first possibility (cf. pl. XV., figs. 2 *a* and 8 *a*).
Fig. 16 *a*. Same, second furrow, second possibility (cf. pl. XV., fig. 2 *a* and 8 *a*).
Fig. 16 *b*. Same, third furrow.
Fig. 16 *c*. Same, fourth furrow.
Fig. 17. Same, egg compressed between vertical plates, first furrow.
Fig. 17 *a*. Same, egg compressed between vertical plates, second furrow.
Fig. 17 *b*. Same, egg compressed between vertical plates, third furrow.
Fig. 18. Same, egg compressed between horizontal plates, first furrow.
Fig. 18 *a*. Same, egg compressed between horizontal plates, second furrow.
Fig. 18 *b*. Same, egg compressed between horizontal plates, third furrow.

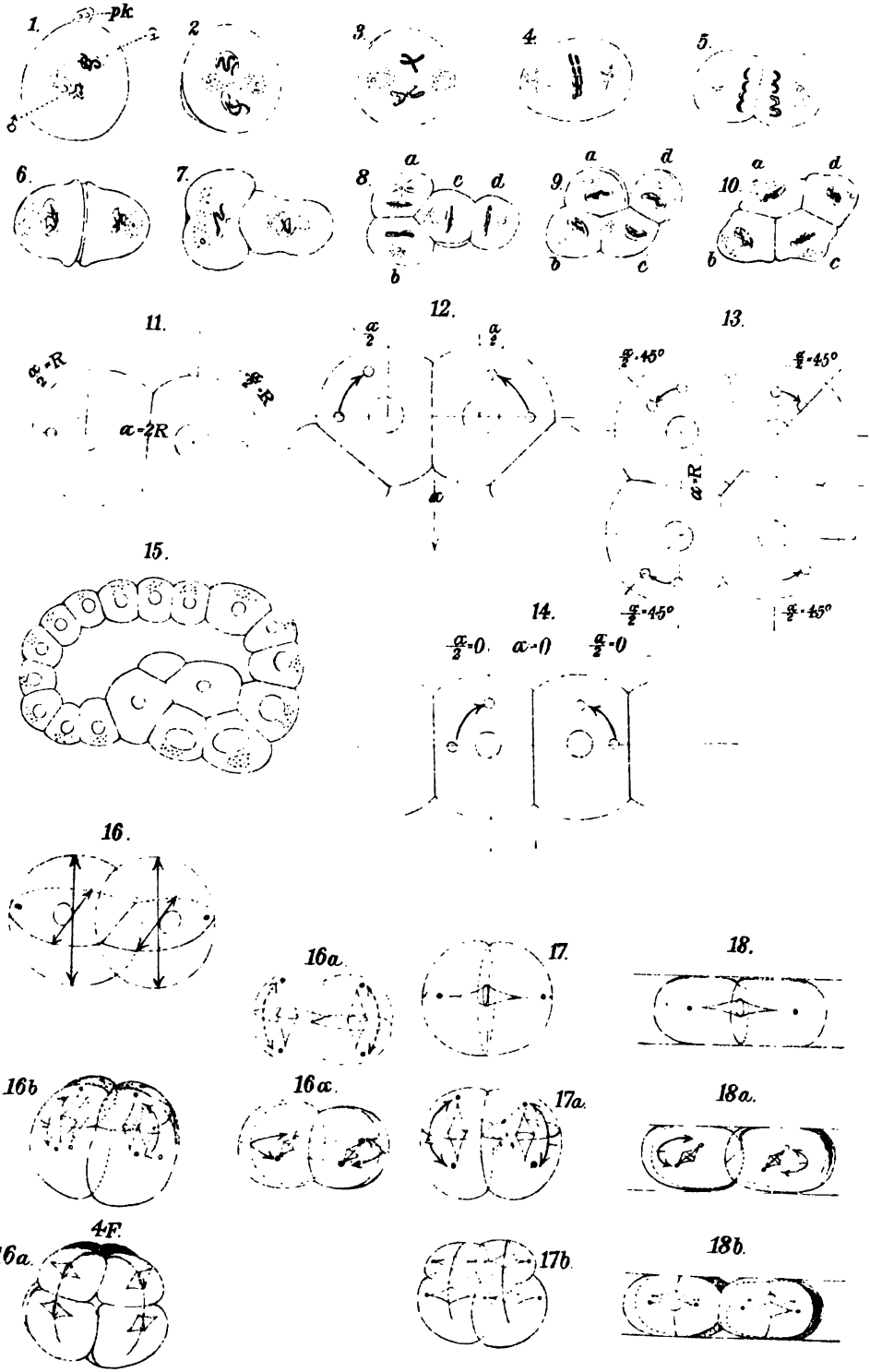


PLATE V.

MITOTIC CELL DIVISION.

2. *Radiation of the plasm.*

Fig. 1. Model illustrating cell-division, after Heidenhain.
Hoop with rubber bands, which are fastened to pegs along the periphery and at their other ends the bands from each half of the periphery converge to two small hoops fastened together; these are drawn apart in 1 *a* by the rubber bands after the thread uniting them has been cut.

Fig. 2. Similar model, with hinges, which in 2 *a* assumes a dumb-bell form after the connecting thread has been cut.

Fig. 3. Diagram representing lines of force between two like and one unlike pole.

Fig. 4. Diagram representing lines of force between three like poles.

Fig. 5. Diagram of the "triaster" figures which occur during nuclear divisions.

Fig. 6. Egg with "triaster" figure (Polyspermy).

Fig. 7—12. Diagrammatic representation of the process of fertilisation and cell division made from drawings of various stages.

Fig. 7. Unfertilised egg and spermatozoon.

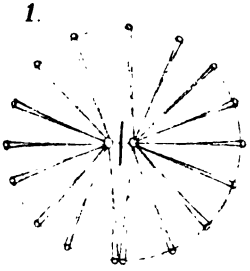
Fig. 8. Union of egg and sperm nucleus, after Wilson.

Fig. 9. Preparation for the first cell division (prophase), after Drüner and Gurwitsch.

Fig. 10. First nuclear division (metaphase), after Ziegler, Wilson, Gurwitsch.

Fig. 11. First cell division (anaphase), after Ziegler.

Fig. 12. Two-cell stage (telophase).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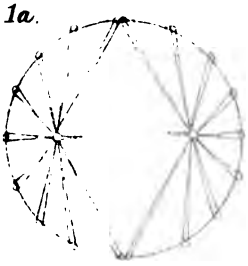
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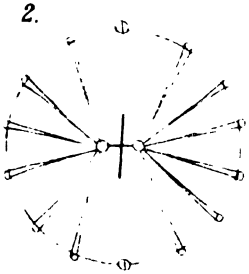
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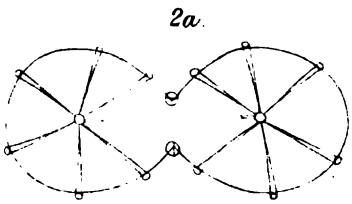
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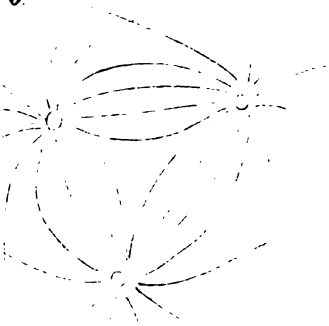
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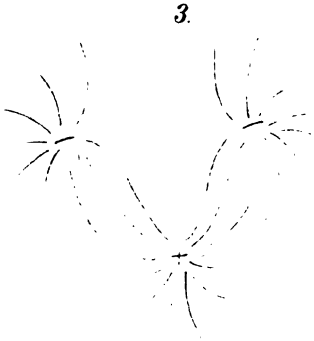
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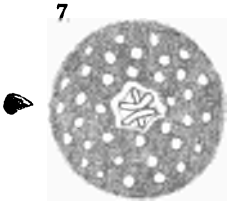
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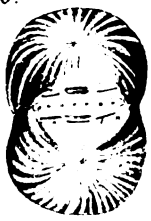
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12.

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PLATE VI.

ARRANGEMENT OF THE CELLS.

Fig. 1. Oil drop floating in a mixture of alcohol and water, just touching the enclosing wine glass with its equator, seen from above; division into two drops, after Roux.

Fig. 2. Same, divided into four, after Roux.

Fig. 3. Same, two quarters again divided into two, after Roux.

Fig. 4 and 4 *a*. Drop, already divided into two, divided into four in different ways, after Roux.

Fig. 4 *a*. Common result of both divisions (4 and 4 *a*), after Roux.

Fig. 5. Circle of 8 oil drops, of which one (*a''*) is bigger than the others, after Roux.

Fig. 5 *a*. Thrusting of the larger drop into the interior, after Roux.

Fig. 5 *b*. Result of the displacement, after Roux.

Fig. 6. Persistence of an open arc of drops if the oil is sufficiently impure (slight surface tension), after Roux.

Fig. 7. Cleavage cells of *Rana fusca* approaching each other in diluted white of egg ("cytotaxis"), "cytotropism" of Roux.

Fig. 8 and 8 *a*. Cleavage cells coming into contact, "cytarme" of Roux.

Fig. 9 and 9 *a*. Gliding of cleavage cells, "cytolisthesis" of Roux.

Fig. 10 and 10 *a*. Two arrangements of pigment in united cells of Roux.

Fig. 11 and 11 *a*. Self division of united cells, "cytochorism" of Roux.

Fig. 11. Self separation of cells by a cleft "cytochorism" of Roux.

Fig. 12 and 12 *a*. Diagram representing formation of the epithelium: plane structure, after Zur Strassen.

Fig. 13 and 13 *a*. Diagram representing formation of the epithelium: bent structure, after Zur Strassen.

Fig. 14 and 14 *a*, 14 *b*. Diagram representing formation of the epithelium: possibility of both structures, after Zur Strassen.

Fig. 15. Diagram representing the polarity conditions of the first two cells, after Zur Strassen.

Fig. 15 *a*. Diagram representing polarity conditions of two cells in epithelial connection, after Zur Strassen.

Fig. 16. Model representing invagination, after Rhumbler. Threaded hoop of steel variously weighted.

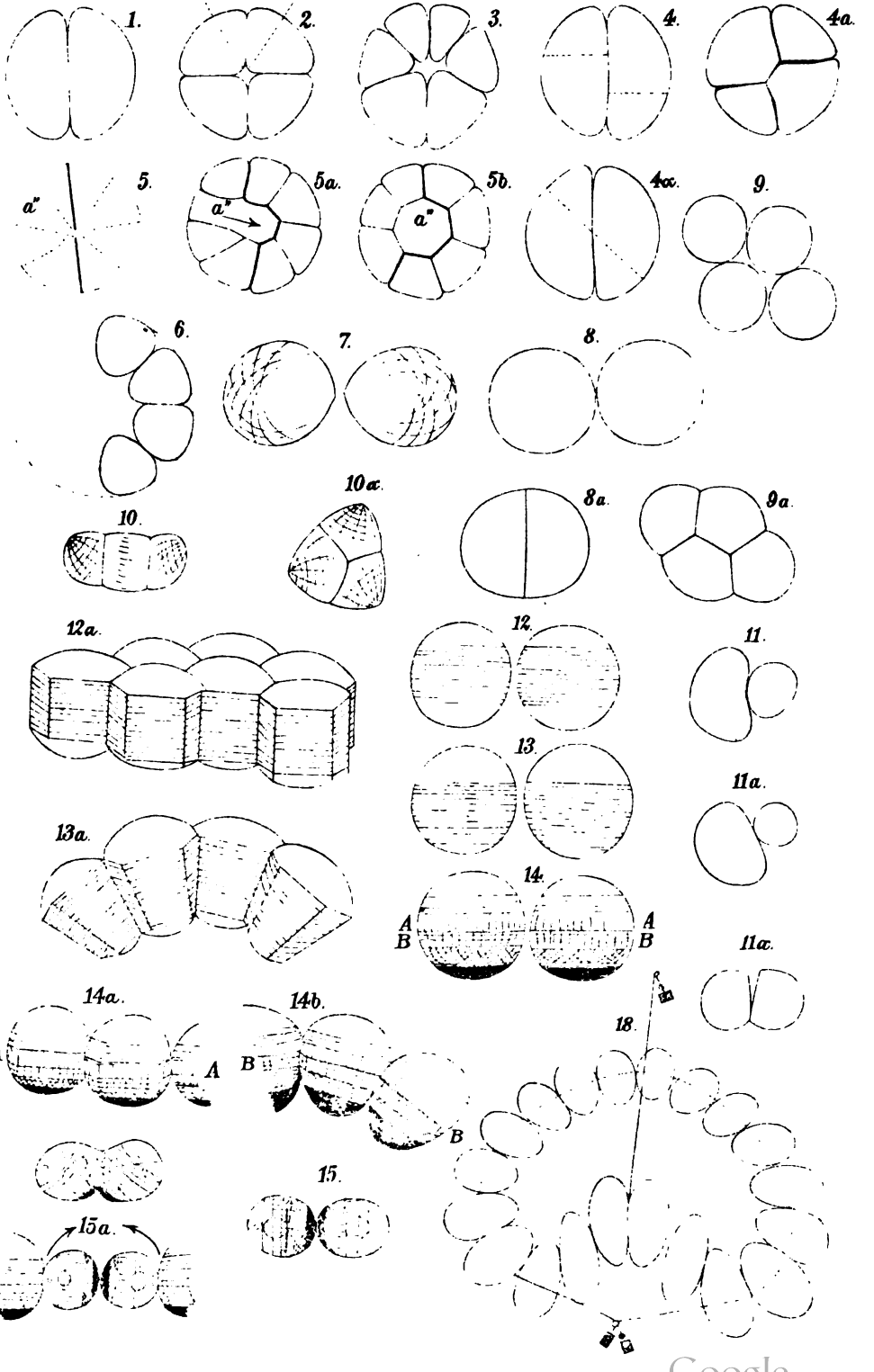

PLATE VII.

MECHANISM OF DEVELOPMENT OF THE CNIDARIA.

Fig. 1 *a—f*. Development of a polyp of *Clytia flavidula* from a cell of the two-cell stage, after Zoja.

Fig. 2 *a—c*. Development of a polyp of *Clytia flavidula* from a cell of the four-cell stage (¼ egg), after Zoja.

Fig. 3. *Aegineta flavescens*, four-cell stage, after Maas.

Fig. 4. *Aegineta flavescens*, equal form of the eight-cell stage, after Maas.

Fig. 4 *a*. Development of half of this form to medusa, after Maas.

Fig. 5. *Aegineta flavescens*, unequal form of the eight-cell stage, after Maas.

Fig. 5 *a*, *b*, *c*. Development when cut up along lines *aa*, *bb*, *cc*.

Fig. 6. *Aegineta flavescens*, displacement of the cleavage cells to form a row, after Maas.

Fig. 6 *a*, *b*, *c*. Development of this form to normal medusa, after Maas.

Fig. 7. Sea-pen (*Renilla*) developed from entire egg, after Wilson.

Fig. 8. Sea-pen (*Renilla*) developed from half egg, after Wilson.

Fig. 9. Sea-pen (*Renilla*) developed from quarter egg, after Wilson.

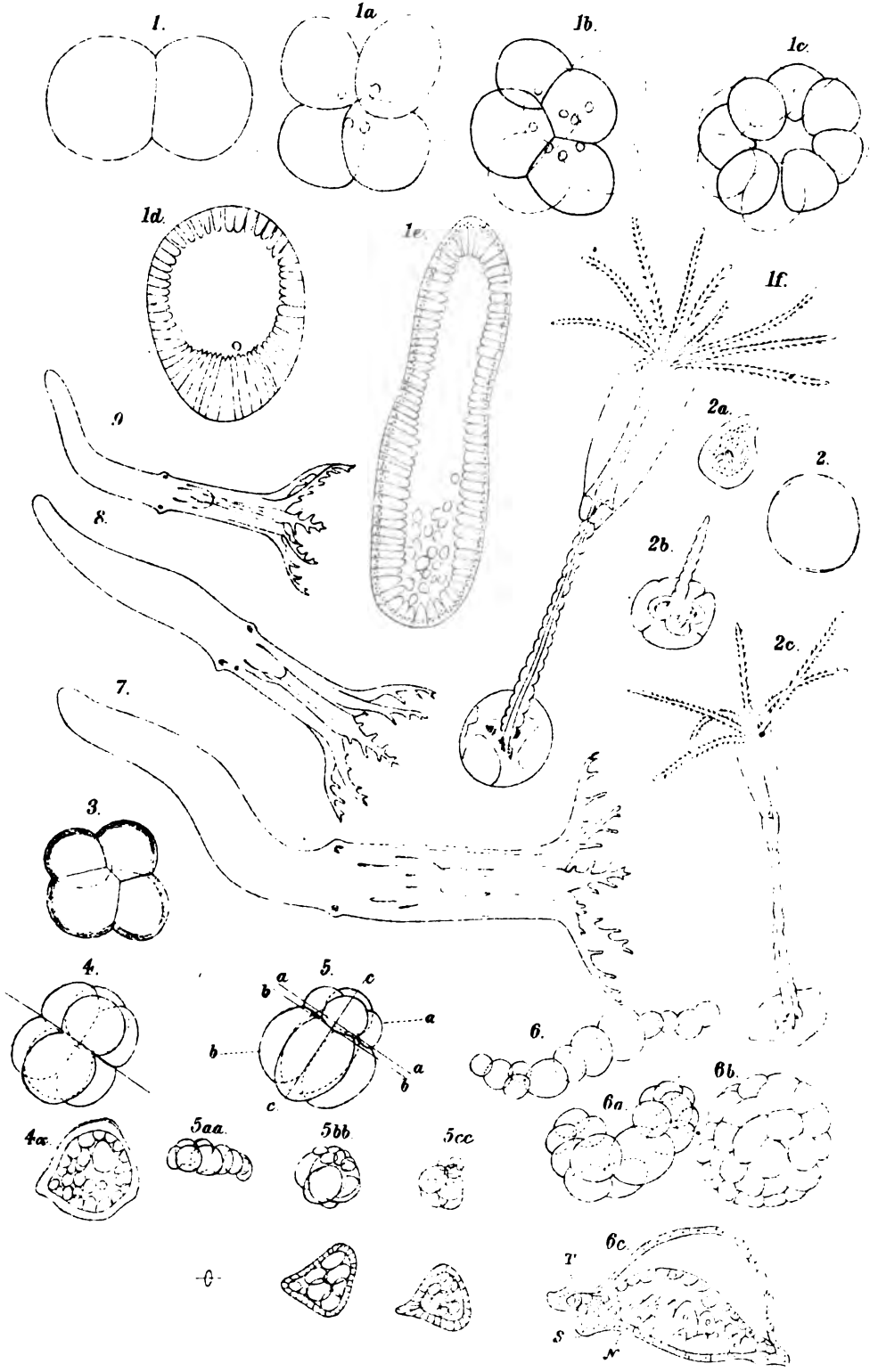

PLATE VIII.

MECHANISM OF DEVELOPMENT OF THE CTENOPHORA.

Fig. 1 *a*—*k*. Development of *Beroë ovata* after Ziegler and Delage; *o* the corresponding stages seen from above. *M* = mouth plane, *T* = tentacle plane (the latter coincides with the plane of the paper for the figures without *o*).

Fig. 2 *a*—*e*. Development of half an egg of *Beroë ovata*, after Driesch and Morgan.

Fig. 3. Displacement of the blastomeres of *Beroë ovata*, after Fischel.

Fig. 3 *a*. Double form developed from this.

Fig. 4. Separation of the blastomeres by pressing the egg membrane between them.

Fig. 4 *a*. (Half) twins obtained from this form, after Fischel.

Fig. 5. Postgeneration during metamorphosis of the missing half of a larva of *Bolina hydatina* obtained from one blastomere of the two-cell stage, after Chun (*r* = regenerated parts: tentacle base and four meridional vessels).

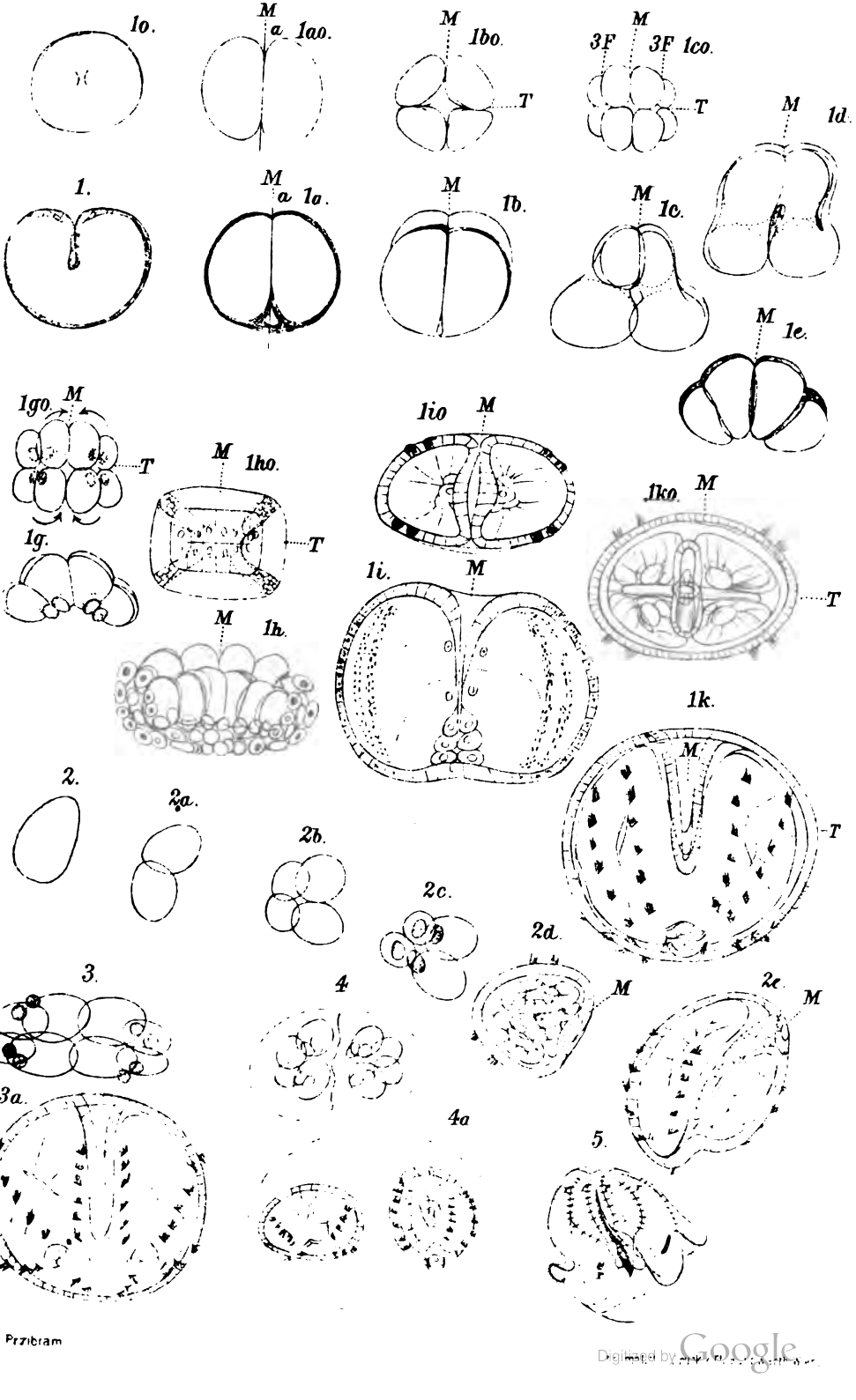

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PLATE IX.

MECHANISM OF DEVELOPMENT IN THE ECHINODERMATA.

Fig. 1—6. Diagrams showing development of the echinoderms. *aa*, *bb*, *cc* sections: after Boveri, Driesch and Roule.

Fig. 1. Sixteen-cell stage of the sea urchin *Strongylocentrotus lividus*. *Ma* = macromeres, *Me* = mesomeres, *Mi* = micromeres.

Fig. 1 *a*. Larva from the vegetative part (below *aa*).

Fig. 1 *c*. Larva from the animal part (above *cc*).

Fig. 2. Larva before the coelom sacs are constricted off.

Fig. 2 *a*. Larva from vegetative part of same (below *aa*).

Fig. 2 *c*. Larva from animal part of same (above *cc*).

Fig. 3. Starfish larva after formation of the coelom sacs (*os* = mouth, *A* = anus).

Fig. 3 *a*. Further development. Part containing the coelom sacs below *aa*.

Fig. 4. Larva after formation of the calcareous triradiate spicules.

Fig. 4 *a*. Further development after one triradiate spicule has been removed (along *bb*).

Fig. 5. Complete "pluteus" larval form of the sea urchin.

Fig. 5 *s*. Same seen from the side (*os* = mouth, *A* = anus).

Fig. 6. Sea urchin after metamorphosis, after Roule.

Fig. 6 *s*. Same seen from the side (*os* = mouth, *A* = anus).

Fig. 7—9. Isolation of blastomeres and their development when reared, after Zoja and Driesch.

Fig. 7. Eight-cell stage of the sea urchin, *Strongylocentrotus lividus*.

Fig. 7 *a*. Isolated vegetative blastomere of same.

Fig. 7 *α*. Further development of same to gastrula.

Fig. 7 *γ*. Isolated animal blastomere of the eight-cell stage.

Fig. 7 *c*. Further development of same to "long-cilia" blastula.

Fig. 8. Four-cell stage of the sea urchin *Strongylocentrotus lividus*.

Fig. 8 *β*. Isolated blastomere of same.

Fig. 8 *b*. Pluteus developed from same.

Fig. 9. Two-cell stage of the sea urchin *Strongylocentrotus lividus*.

Fig. 9 *b*. Pluteus from an isolated blastomere of the two-cell stage.

Fig. 10. Eight-cell stage of the sea urchin under pressure, after Driesch.

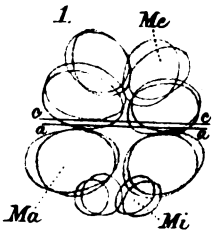
Fig. 10 *a*. Further development after the pressure is relaxed.

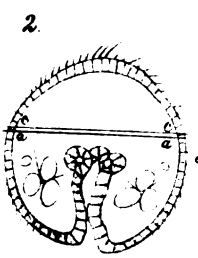
Fig. 10 *b*. Normal pluteus obtained from this form (outline).

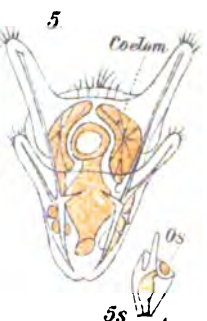
Fig. 11. "Extraovates" which have burst forth and are continuing to develop, after Loeb.

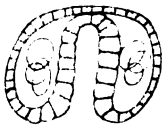
Fig. 12. Giant pluteus developed from two eggs which have coalesced, after Driesch.

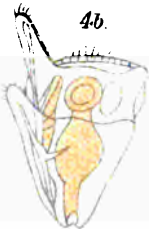
Fig. 13. Sea urchin morula obtained from transplanted blastomeres of different colour, after Garbowski. The blastomeres marked with an orange circular spot belong to the vegetative half of the egg.

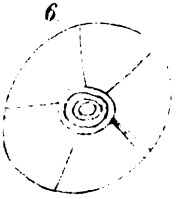


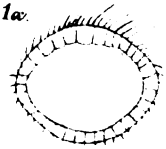


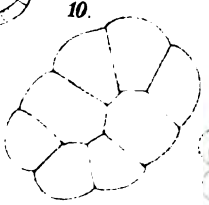


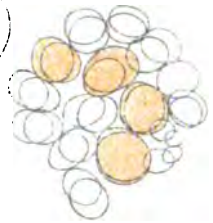


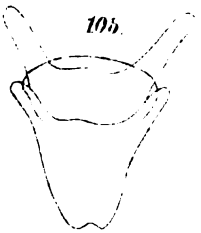


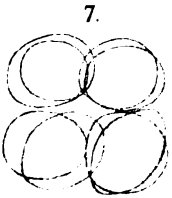


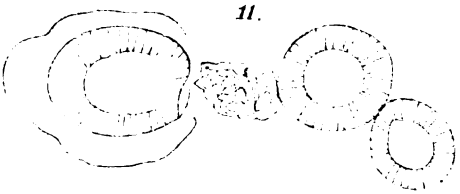


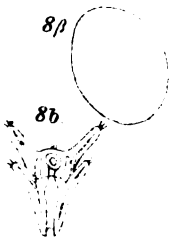


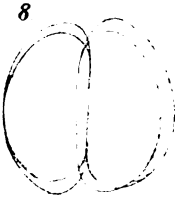


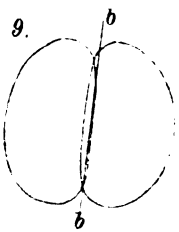


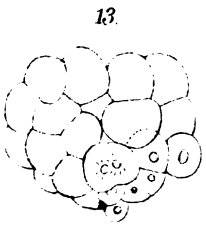












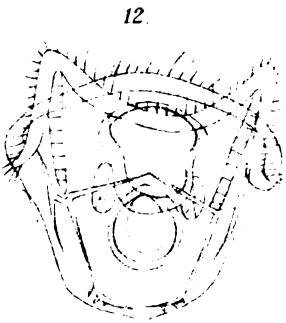


PLATE X.

MECHANISM OF DEVELOPMENT OF THE NEMATODA.

Fig. 1. Two-cell stage (ventral view) of *Ascaris megalocephala*, after Zur Strassen.

Fig. 2. Four-cell stage (ventral view) of *Ascaris megalocephala*, after Zur Strassen.

Fig. 3. Eight-cell stage (ventral view) of *Ascaris megalocephala*, after Zur Strassen.

Fig. 3 *s*. Eight-cell stage (from the side) of *Ascaris megalocephala*, after Zur Strassen.

Fig. 4. Later stage (ventral view) of *Ascaris megalocephala*, after Zur Strassen.

Fig. 5. Embryo (ventral view) of *Ascaris megalocephala*, after Zur Strassen.

Fig. 5 *s*. Embryo (from the side) of *Ascaris megalocephala*, after Zur Strassen.

Fig. 6. Worm of *Ascaris megalocephala* (ventral view) ready to emerge, after Zur Strassen.

The egg envelope is omitted in figs. 1—6. The large heavy nuclei are the undiminished nuclei which in the later stages remain only in the sexual zone (Boveri).

Fig. 7. Giant egg of *Ascaris megalocephala*, after Zur Strassen.

Fig. 8. T-shaped giant developed from two eggs, attempting in vain to produce a normal four-cell stage.

Fig. 9. Division of a threefold giant by an elongated constriction of the egg envelope.

Fig. 10 *a*. Further development of the larger cell complex.

Fig. 10 *b*. Further development of the smaller, purely ectodermal cell complex.

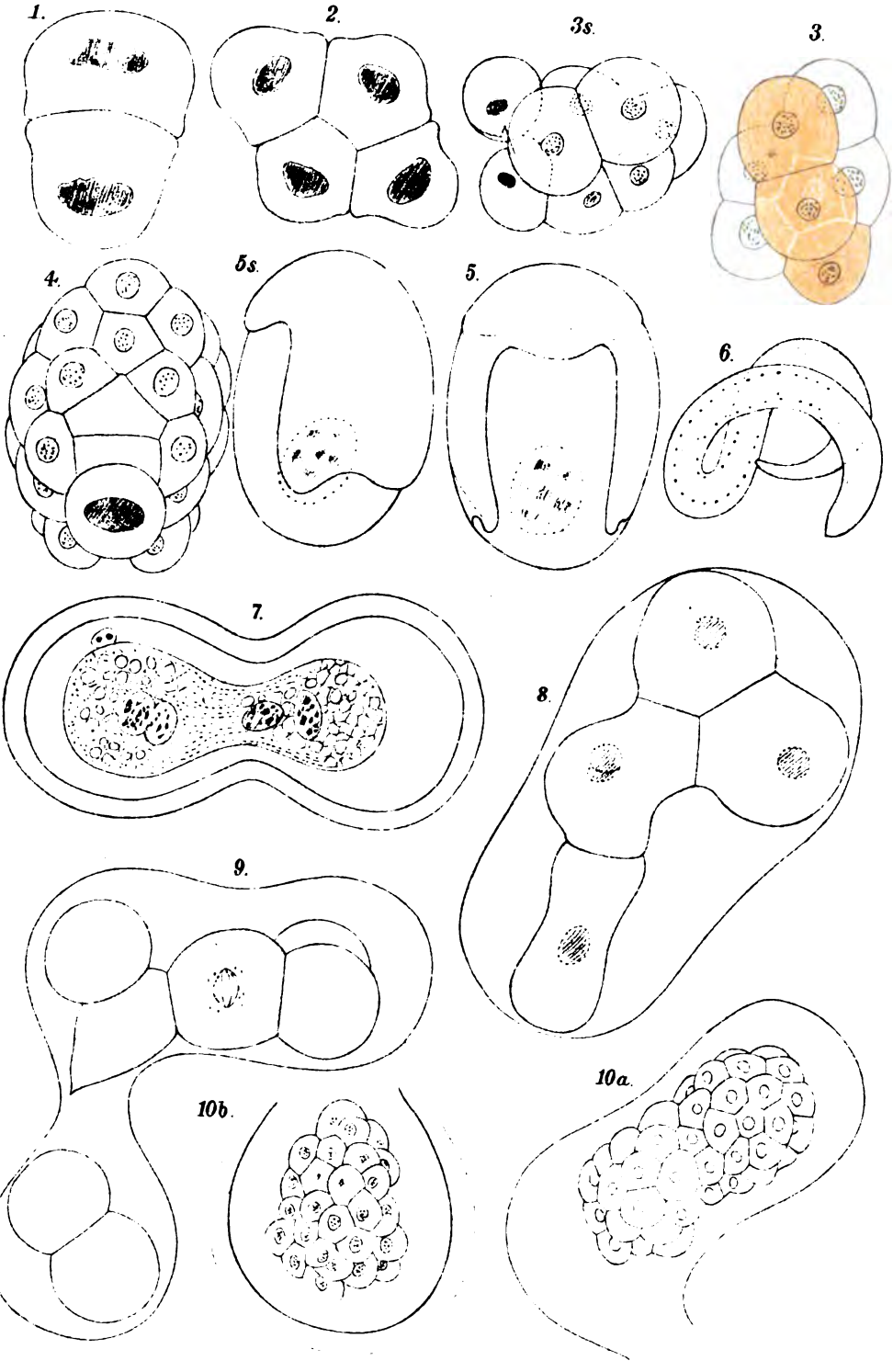


PLATE XI.

MECHANISM OF DEVELOPMENT IN THE NEMERTINA AND ANNELIDA.

Fig. 1. Egg of the nemertine *Cerebratulus lacteus*, after Yatsu.

Fig. 1 *a*. Egg of the nemertine *Cerebratulus lacteus* during incision of the first furrow.

Fig. 1 *b*—*d*. "Pilidia" from the eggs cut in two along $\gamma\gamma$.

Fig. 2. Egg of the nemertine *Cerebratulus marginatus*, after Zeleny. Two-cell stage.

Fig. 2 *a*. Further development of same to gastrula.

Fig. 2 *b*. Four-cell stage from an isolated blastomere of the two-cell stage.

Fig. 2 *b'* and 2 *bb''*. Further development of same.

Fig. 3. Egg of the nemertine *Cerebratulus marginatus*, four-cell stage.

Fig. 3 *b*. Further development of an isolated blastomere of same.

Fig. 4. Egg of the nemertine *Cerebratulus marginatus*, eight-cell stage.

Fig. 4 *a*—*c*. Further development after the operations indicated by *aa*, *bb*, *cc*.

Fig. 5. Egg of the nemertine *Cerebratulus lacteus*, blastula, after Wilson.

Fig. 5 *a*—*c*. Further development after division along lines *aa*, *bb*, *cc*; *c* and *aa* from the lower (vegetative) half; *cc* and *a* from the upper (animal) half.

Fig. 6. Egg of the annelid Nereïs, four-cell stage, after Wilson.

Fig. 6 *s*. Egg of the annelid Nereïs, seen from the side, after Wilson.

Fig. 6 *a*. Eight-cell stage of the Nereïs egg, after Wilson.

Fig. 6 *b*. "Trochophore" stage, after Wilson.

Fig. 6 *c*. Nine and twenty-cell stage, after Wilson.

Fig. 7 *a*. Eight-cell stage produced by pressure from the egg, fig. 6, after Wilson.

Fig. 7 *b*. Sixteen-cell stage formed after removal of pressure, after Wilson.

Fig. 7 *c*. Trochophore from same with eight instead of four endodermal cells (yellow).

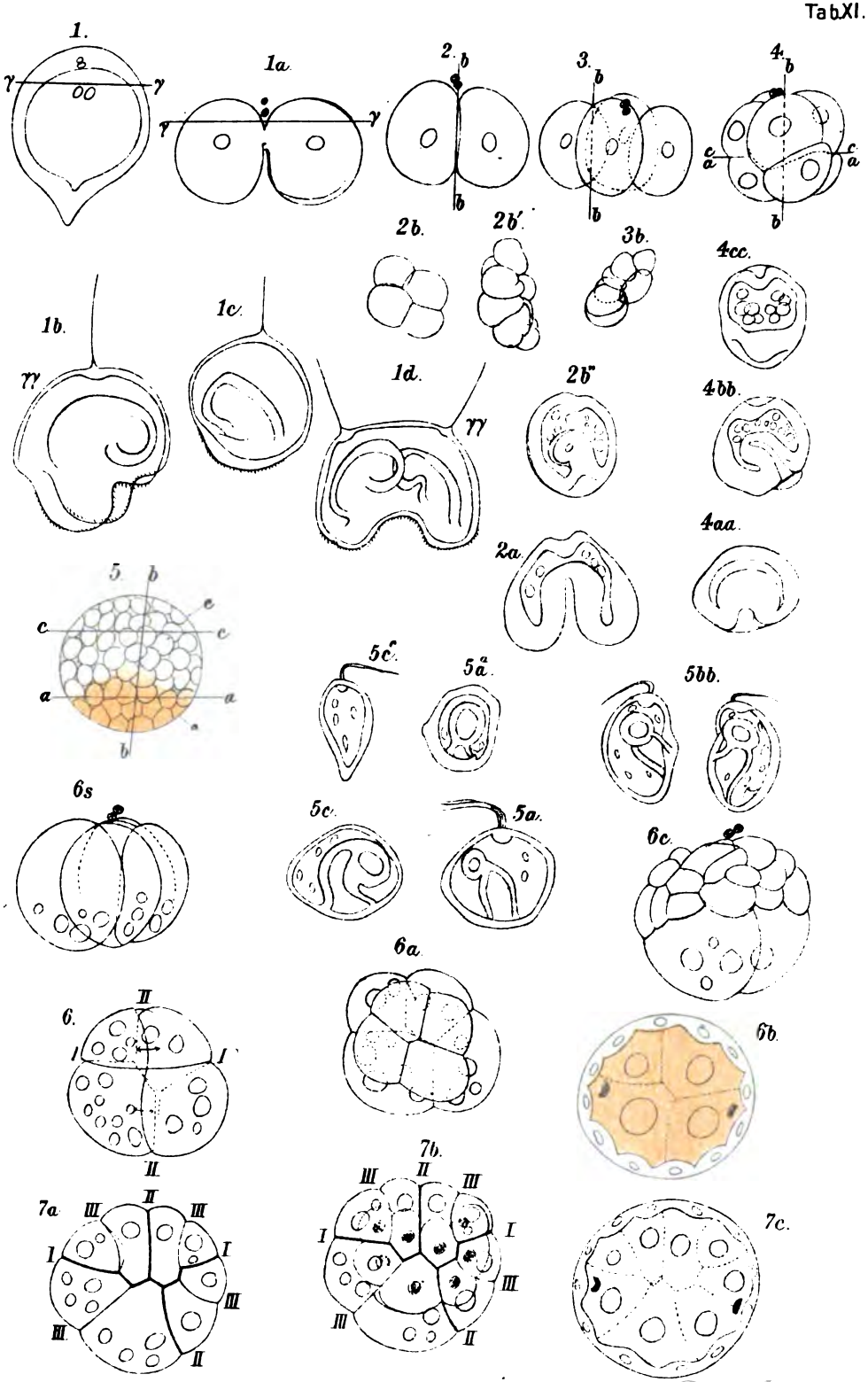


PLATE XII.

MECHANISM OF DEVELOPMENT OF THE MOLLUSCA.

A. Dentalium.

Fig. 1—10. Development of Dentalium.

Fig. 1. Fertilised egg before the commencement of the first division (1 hour after impregnation), after Wilson.

Fig. 2. Beginning of the first division, formation of the "yolk-lobe" (Dl_1).

Fig. 3. During the first division, "trefoil" form, 1½ hours after impregnation.

Fig. 4. Complete two-cell stage.

Fig. 5. Second division (culmination).

Fig. 6. Eight-cell stage (after Crampton for Ilyanassa).

Fig. 7. Sixteen-cell stage (after Crampton for Ilyanassa) (The "primary trochoblasts" punctuated).

Fig. 8. Trochophore of Dentalium, after Wilson.

Fig. 9. Metamorphosis of same, after Wilson.

Fig. 10. Young metamorphosed Dentalium, after Wilson.

The small figures are diagrams of the further development of parts of the egg isolated by cutting along *aa*, *aa*, *bb*; the numbers refer to the stage operated upon which is drawn under the corresponding number without letters; the letters refer to the parts of the egg which have attained to further development; they are marked in the normal development by placing the letters in the parts themselves.

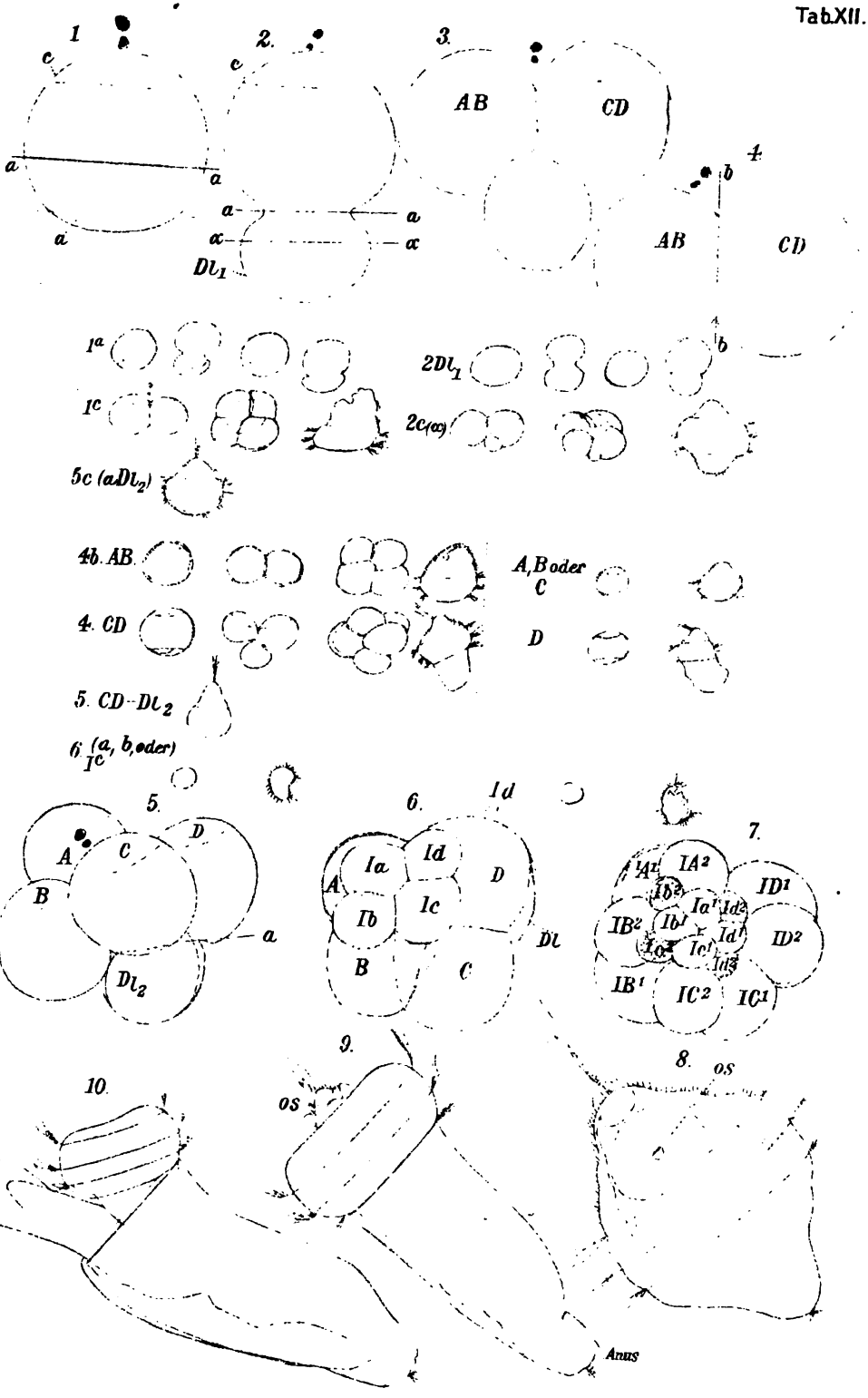


PLATE XIII.

MECHANISM OF DEVELOPMENT OF THE MOLLUSCA.

B. Patella.

Fig. 1. Egg of Patella (rock-limpet), after Wilson.
Four-cell stage from above, after Wilson.

Fig. 2. Sixteen-cell stage of same from above, after Wilson.

Fig. 3. Fifty-two cell stage of same from above, after Wilson.

Fig. 4. Later stage of same, sagittal optical section, after Wilson.

Fig. 5. So-called "ctenophore-stage," from above, after Wilson.

Fig. 6. Trochophore, seen from the left side, after Wilson.

Fig. 7 *b*. Closed type of development of an isolated blastomere of the two-cell stage, from above, after Wilson.

Fig. 7 *β*. Open type in same operation, seen from above, after Wilson.

Fig. 8. Closed larva from isolated blastomere of the two-cell stage, from above.

Fig. 9. Further development of an isolated blastomere of the four-cell stage, from the side.

Fig. 10. Open larva from isolated blastomere of the two-cell stage, from the side.

Fig. 11. Isolated micromere of the eight-cell stage, from above.

Fig. 12. Further development of same.

Fig. 13. Isolated macromere of the eight-cell stage.

Fig. 14. Next division of same.

Fig. 15. Further development of same, from the side.

Fig. 16. Isolated macromere of the sixteen-cell stage.

Fig. 17. Further development of same, from the side.

Fig. 18. Isolated primary trochoblast and further development.

Fig. 19. Isolated cell of the next division of the trochoblast and further development.

Fig. 20. Isolated cell of the second next division of the trochoblast and further development.

Fig. 21. Isolated sister-cell of the primary trochoblast and further development.

Fig. 22. Apical cell developed singly.

Fig. 23. Secondary trochoblast developed singly.

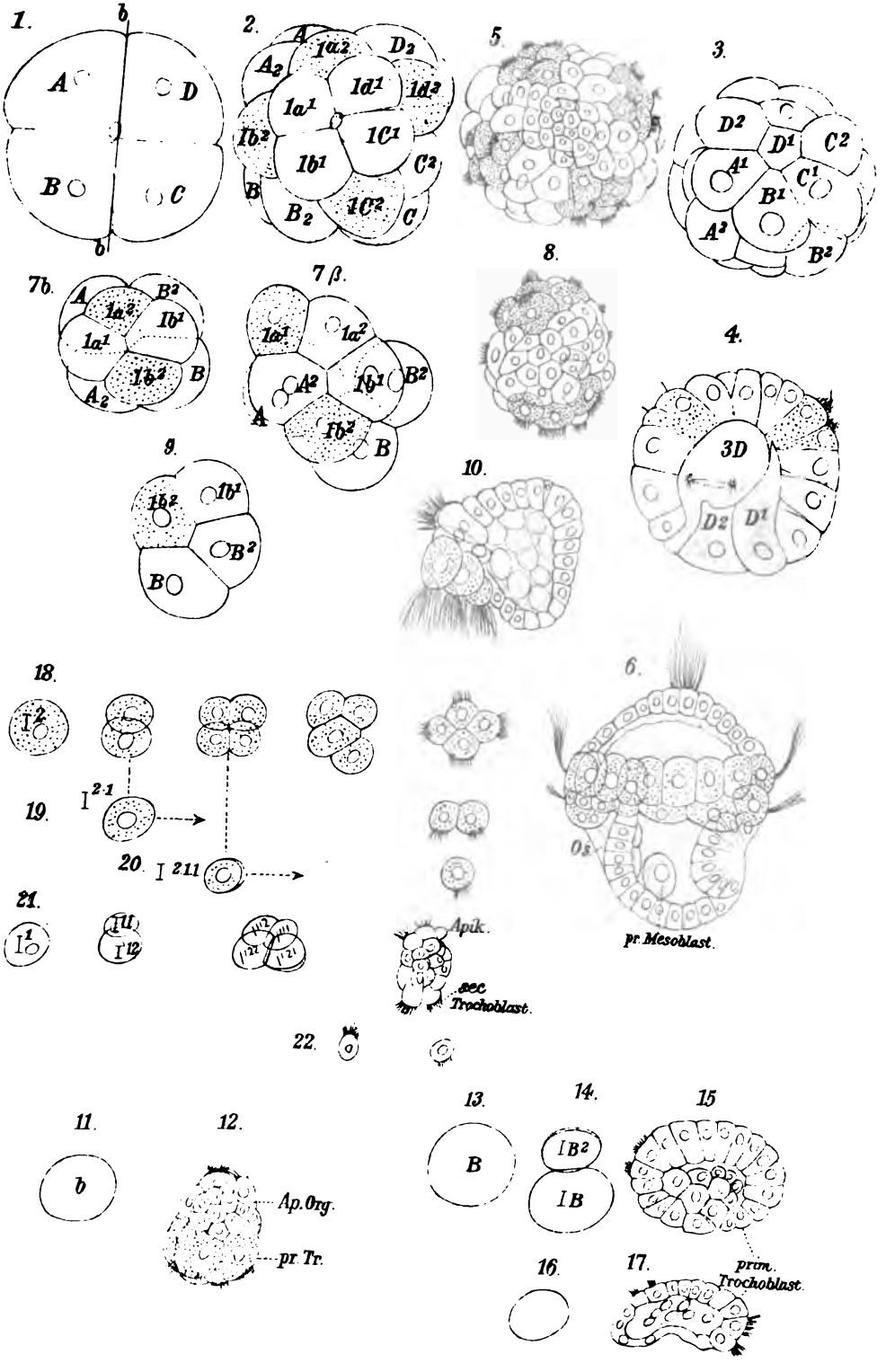


PLATE XIV.

MECHANISM OF DEVELOPMENT OF THE PROCHORDATA AND PISCES.

Fig. 1—7. Normal development of the Ascidia, after Wilson and Delage.
Fig. 1 *s*. Fertilised egg, from the side.
Fig. 2. Two-cell stage, from above.
Fig. 2 *s*. Two-cell stage, seen from the side.
Fig. 3. Four-cell stage, from above.
Fig. 3 *s*. Four-cell stage, seen from the side.
Fig. 4. Later (blastula ?) stage.
Fig. 5 *s*. Gastrula, from the side (longitudinal section).
Fig. 6 *s*. Later stage (longitudinal section).
Fig. 7 *s*. Perfect larva (longitudinal section).
Fig. 8. Half larva developed from two isolated cells of the four-cell stage of the ascidia *Ascidiella aspersa*, after Chabry.
Fig. 9. Larva developed from isolated blastomere of *Phallusia mammillata*, after Driesch.
Fig. 10. Egg of *Amphioxus lanceolatus* after fertilisation, Yves Delage.
Fig. 10 *a*. Gastrula developed from same (cross section), after Yves Delage.
Fig. 11. Two-cell stage.
Fig. 11 *a*. Gastrula derived from isolated blastomere of same (cross section), after Wilson and Morgan.
Fig. 12. Four-cell stage, from above, after Wilson and Morgan.
Fig. 12 *s*. Four-cell stage seen from the side, after Wilson and Morgan.
Fig. 12 *a*. Gastrula derived from isolated blastomere of same, after Wilson and Morgan (cross section).
Fig. 13. Double blastula of Amphioxus (cross section), after Wilson and Morgan.
Fig. 14—16. Egg of lamprey *Petromyzon planeri*, with twin embryos produced by "blastotomy," after Bataillon.
Fig. 17. Germinal rim from the egg of Scyllium, after Kopsch.
Fig. 18. Result of operation indicated by the line in fig. 17.
Fig. 19. Eggs of *Amia calva* on a leaf, after Eycleshеimer.
Fig. 20. Cleavage of the egg of *Amia calva*, Ist—IIIrd furrow, after Eycleshеimer.
Fig. 21. Cleavage of the egg of *Amia calva* from the upper pole, after Eycleshеimer.
Fig. 21 *a—d*. Position of the embryos when that of the Ist, or Ist and IInd furrows is fixed, after Eycleshеimer.
Fig. 22—26. Diagrammatic representation of the development of the bony fish, after Sumner.
Fig. 27—29. Determination of the regions in which the embryo arises by inserting bristles in certain parts of the egg of Fundulus, after Sumner.
Fig. 30. Embryo of Fundulus from a cell of the two-cell stage, after Morgan.
Fig. 31. Later development of a trout embryo (*Truttio fario*) after an operation similar to that indicated in fig. 17, after Kopsch.
Fig. 32—34. Monstrous multiple forms of the roach *Leuciscus rutilus* after immersion in salt solutions, after Bataillon.

PLATE XV.

MECHANISM OF DEVELOPMENT OF THE TETRAPODA.

Fig. 1. Egg of Triton before cleavage, from above, after Spemann.

Fig. 2 *a*. Two-cell stage of same, rare case of the first furrow in the later median plane (2 *b*) from above, after Spemann.

Fig. 2 *c*. Development of two embryos from same, after Herlitzka.

Fig. 2 *a*. Two-cell stage of the Triton egg, more frequent case of the first furrow in the future frontal plane (2 β), from above, after Spemann.

Fig. 2 γ and 2 δ. Further development of a single (dorsal) fragment to perfect embryo, after Spemann.

Fig. 3 *a*. Development of the two halves of Triton embryo constricted across in the late gastrula stage, after Spemann.

Fig. 3 β. Further development of the fore fragment (above) with brain (*cr*) and primary eye vesicles (*oc*), and of the hind fragment (below) with medullary groove (*me*), after Spemann.

Fig. 4, 5. Embryos of *Triton taeniatus* with extreme duplication of the fore end, after Spemann.

Fig. 6. *Triton taeniatus*, cross section of the head of an embryo: the primary eye vesicle (*oc*) of the left side about to be converted into the eye cup; lens rudiment not yet visible; on the right the primary cells for lens formation (and a part of the future eye cup) have been removed, after Spemann.

Fig. 6 *a*. On the left invagination of the lens (*l*); on the right lens formation has been prevented by the thrusting in of the mesoderm (*me*), after Spemann.

Fig. 6 *b*. On the left the lens vesicle has been constricted off from the epidermis; on the right a lens beginning to invaginate since in this case (in contrast to 6 *a*) the eye cup had reached the epidermis, after Spemann.

Fig. 7. Egg of *Rana esculenta*, from above, after Roux. (The straight lines indicate that the corresponding blastomere has been pricked.)

Fig. 8 *a*. Two-cell stage of same, from above, after Roux.

Fig. 8 *b*. "Semimorula" developed from same, from above, after Roux.

Fig. 8 *c*. "Hemiembryo lateralis" developed from same, from above, after Roux.

Fig. 8 *a*. Two-cell stage with second furrow differently placed, after Roux.

Fig. 9 *a*. Four-cell stage developed from same, after Roux.

Fig. 9 β. "Hemiembryo anterior" developed from same, after Roux.

Fig. 10. Double-headed frog embryo after Schultze's experiment.

Fig. 11. Diagram representing development of same from the side, after Wetzel.

Fig. 12. Diagram representing development of a diminished perfect form from one blastomere, in Morgan's experiment of pricking one blastomere (dotted), the white pole turned upwards, from the side.

Fig. 13. Diagram representing development of a hemiembryo in Morgan's experiment, similar to 12 but with the black pole turned upwards, from the side.

Fig. 14. *Rana silvatica* embryo on completion of passive stage from the left side, *oc* eye cup, the cross marks place of ear-operation, after Streeter.

Fig. 14 *a*. Frog developed from the operated embryo; head from above; on the left the ear protuberance is missing, after Streeter.

Fig. 15. *Rana esculenta*, larva with line marked along which brain is to be removed, from the side, after Schaper.

Fig. 15 *a*. Development of larva on which this operation is performed, from above, after Schaper.

Fig. 15 *a*. Development of a normal check larva, from above, after Schaper.

Fig. 16. Rana embryo with line of operation *ab* for removal of dorsal half of the ganglionic crest from the left side, after Harrison.

Fig. 16 *a*. Part of the further developed embryo without sensitive nerves. *Sp C*= spinal ganglionic cord, *u*=abdominal muscles, *H*=rudiment of hind leg, from the left side, after Harrison.

Fig. 17. Rana embryo with lines of operation $a_1 b$, $a_2 b$ for removal of the ventral half of the ganglionic cord, from the left side, after Harrison.

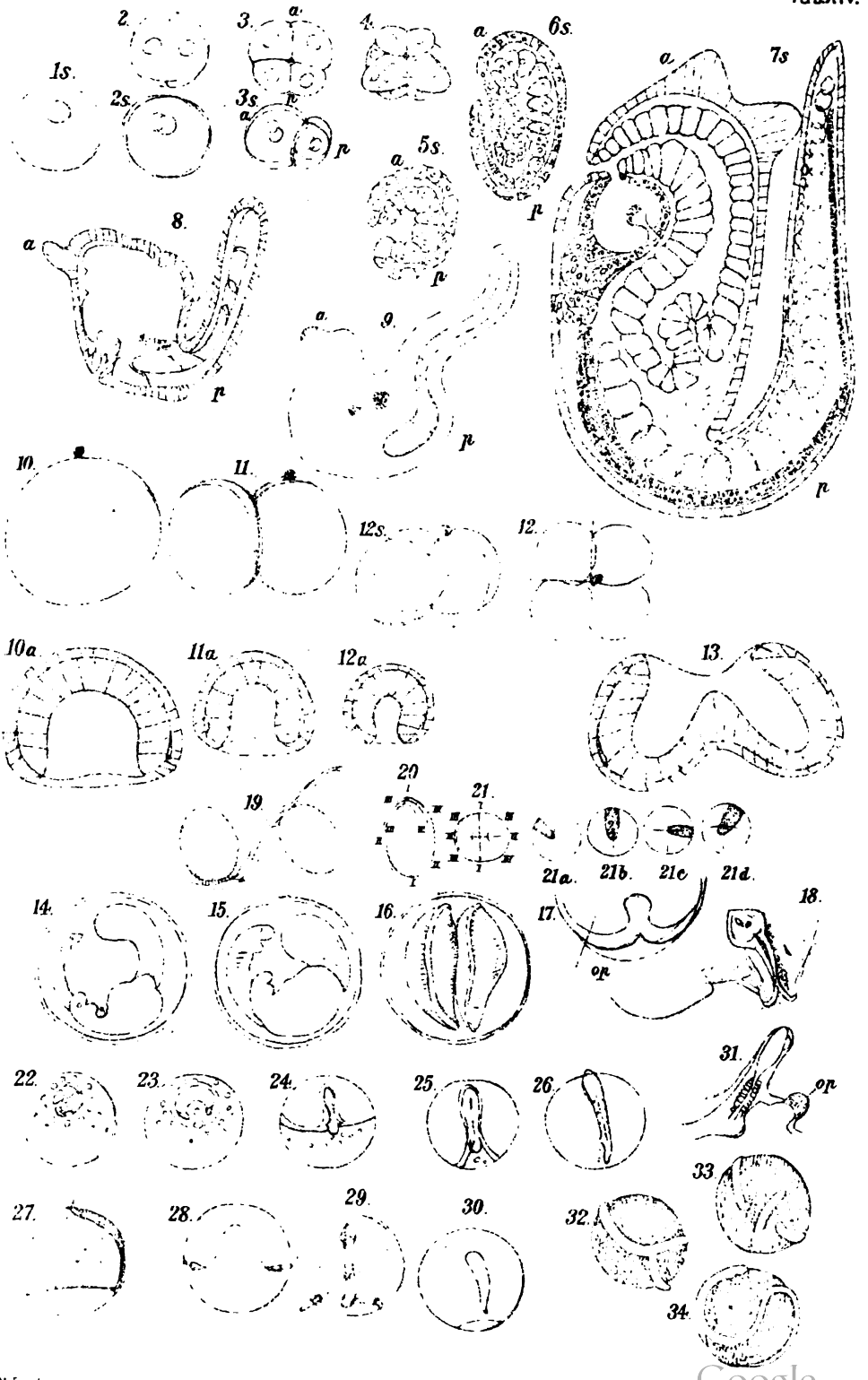
Fig. 17 *a*. Part of the embryo developed further without motor nerves; *Sp C*= spinal ganglionic cord, from the left side, after Harrison.

Fig. 18. Rana larva, normal check specimen with sensitive nerves (yellow) and motor nerves (black) from the left side, after Harrison.

Fig. 19. Egg of the common fowl, *Gallus domesticus*, recently laid; pricked in the exposed germinal sheath, seen from above, after Peebles.

Fig. 19 *a*. Germinal sheath with primitive duct seen from above, after Peebles.

Fig. 19 *b*. Embryo developed further on both sides of the mark, *A* fore end, *O* hind end, after Peebles.



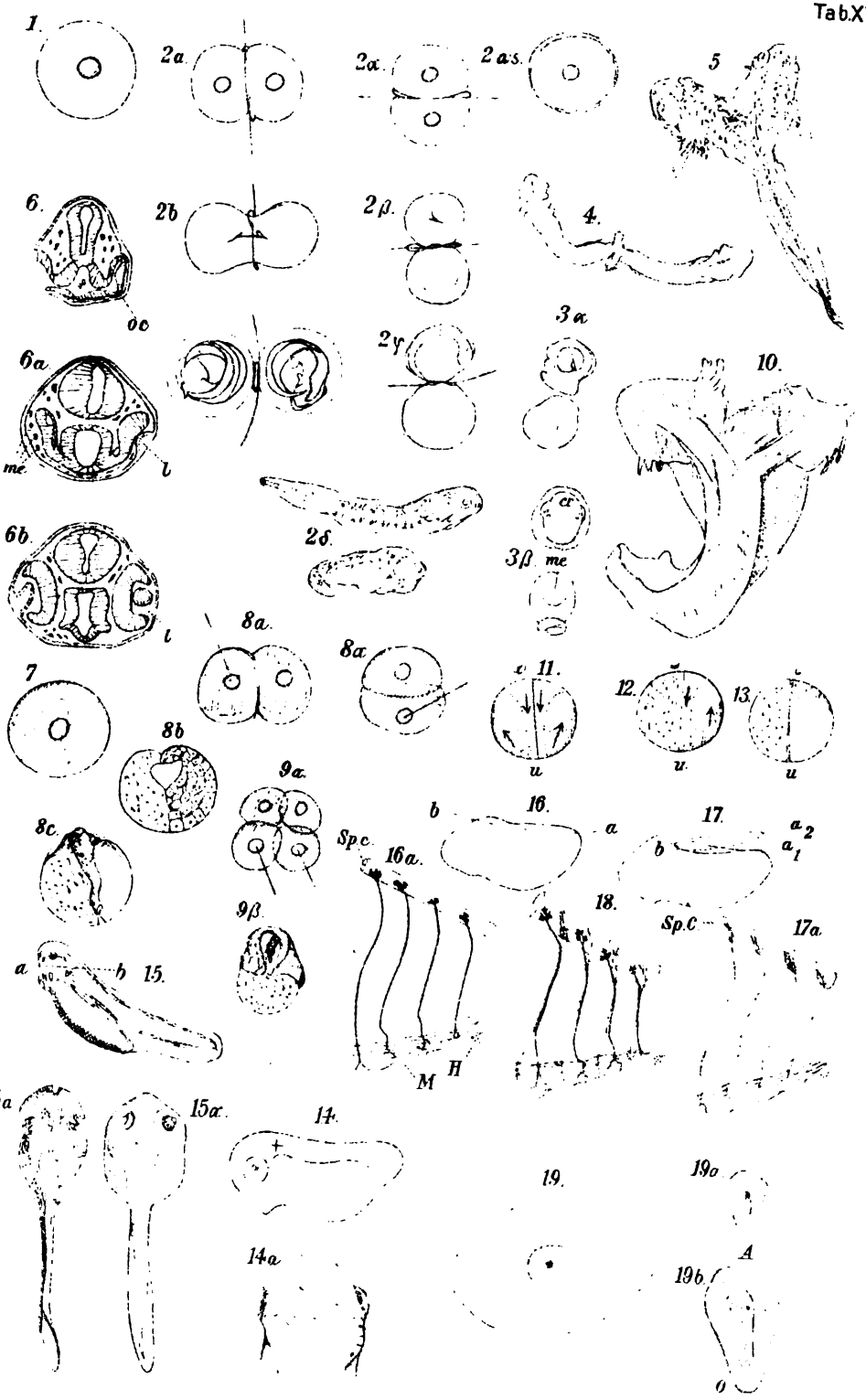


PLATE XVI.

INFLUENCE OF EXTERNAL FACTORS ON DEVELOPMENT.

Fig. 1. Normal sea-urchin larva (pluteus), after Herbst.

Fig. 2. Sea-urchin blastula from sea-water devoid of potassium, after Herbst.

Fig. 3. Sea-urchin larva from sea-water devoid of sulphur, after Herbst.

Fig. 4. Sea-urchin larva from sea-water devoid of sulphates, after Herbst.

Fig. 5. Sea-urchin larva from sea-water devoid of calcium, after Herbst.

Fig. 6. Sea-urchin larva from sea-water containing lithium, after Herbst.

Fig. 7. "Exogastrula" from sea-urchin eggs developed in warmth, after Driesch.

Fig. 8—10. Development of the calcareous sponge Sycandra, after Maas.

Fig. 8. Separation of gastral cells (yellow) and dermal cells (dotted), seen from the side.

Fig. 9. "Amphiblastula," longitudinal section, after Maas.

Fig. 10. Small calcareous sponge (*Sycandra setosa*), general view, after Maas. The round black spots represent pores.

Fig. 11. *Sycandra setosa*, reared in sea-water devoid of carbonates, after Maas.

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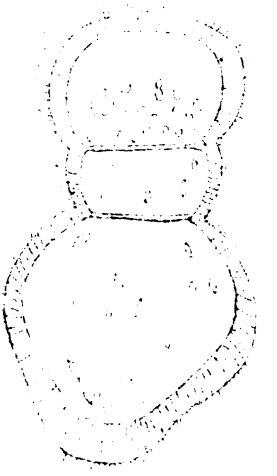

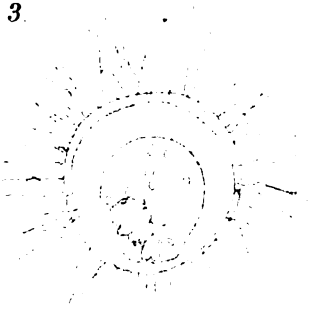

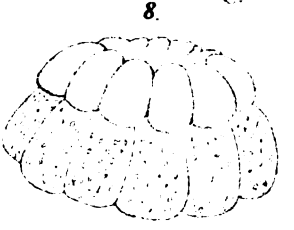

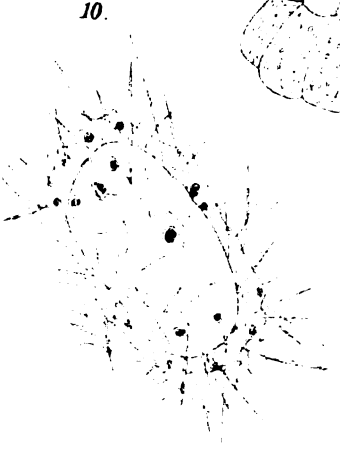

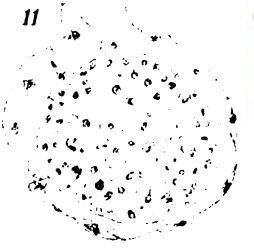

H. Przibram